Physiology of Sleep-Wake Regulation in Health and Disease: A Molecular Imaging and a Clinical Study

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von

Katharina Hefti

von

Luchsingen GL

Promotionskomitee

Prof. Dr. med. Urs Boutellier (Vorsitz)
Prof. Dr. Hans-Peter Landolt (Leitung der Dissertation)
Prof. Dr. med. Claudio Bassetti
PD Dr. Huub van Hedel (Gutachter)

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NEVER

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GIVE

UP

Winston Churchill 1874-1965

Summary

Sleep is ubiquitous among species. Despite this known fact, the behavioral characteristics of sleep and wake are very diverse and species dependant. Sleep behavior manifests itself in many different ways, such as having only one eye closed during sleep (duck), having only one hemisphere in deep sleep at one time (dolphin), having an apnoea during the sleep period (seal), sleeping at different times of the day as seen in nocturnal species (bat), having a polyphasic sleep pattern (mice), or finally, the monophasic sleep pattern that characterizes normal human sleep. Although much remains unknown about the function of sleep, we believe that sleep must serve a vital function since it was conserved in evolution. Therefore, sleep may be essential for the existence of all creatures.

This work focuses on sleep-wake regulation in humans. The study of sleep is of great interest since we are not able to influence consciously the characteristics of this state. Sleep is a state of diminished awareness and reduced responsiveness.

The complexity of sleep makes it an interesting topic of investigation. Sleep states, sleep architecture, sleep timing and duration, and sleep homeostasis are only a few aspects which can be examined. The different characteristics of brain waves during sleep and wakefulness are of great importance in sleep research.

Delta activity is a marker of the sleep electroencephalogram on which the well-known and widely accepted two-process-model of sleep-wake regulation is based. The model hypothesizes that the timing and duration of sleep and waking are the result of two interacting processes. The homeostatic process is dependant on the prior duration of wakefulness, whereas the circadian process is driven by the suprachiasmatic nucleus in the hypothalamus. In addition, the synaptic homeostasis hypothesis, which posits that wakefulness is associated with synaptic potentiation and sleep is associated with synaptic downscaling, served as the theoretical foundation of this thesis.

The **aim of this project** was to study the mechanism of the homeostatic process of sleep on a cellular level by studying the effects of sleep deprivation on metabotropic glutamate receptor subtype 5 (mGluR5) in healthy volunteers. Further, We studied the homeostatic process on a physiological level in patients with idiopathic hypersomnia (IH). We challenged healthy subjects and patients with a period of 40h of prolonged wakefulness.

The **first project** of this thesis (Chapter 2) focuses on the effect of prolonged wakefulness and the availability of mGluR5 in healthy male volunteers, and on possible associations with cognitive functions. This positron emission tomography (PET) study was conducted in a randomized, crossover fashion with the highly selective radioligand ^{11}C-ABP688. Twenty-two male volunteers completed the study, including one control PET scan after 9 hours awake and a sleep deprivation scan after 32 hours of wakefulness (i.e., after spending one night without sleep). The main finding of this study was a global increase in mGluR5 availability after one night without sleep. In addition, a significant increase was measured in six regions of interest that were previously suggested to be involved in sleep-wake regulation. The increase in mGluR5 availability was positively correlated with increase in subjective sleepiness.

The **second project** of this thesis (Chapter 3) aimed to gain a broader understanding of the pathophysiology of IH. By keeping patients with IH awake for 40 hours, we examined homeostatic and circadian aspcects of sleep-wake regulation in these patients. The study was conducted in a matched-pair design with healthy controls. Visually scored sleep states were not statistically different between the two groups in baseline and recovery nights. In addition, non-rapid eye movement sleep (NREMS) episode analysis revealed a similar time course in δ-power decay (0.75-4.5 Hz) in IH patients and healthy volunteers. Nevertheless, we found differences between the groups in EEG θ-/α-, σ- and β-activity. Patients with IH had a significant decrease in power compared to controls in NREMS and REMS. During prolonged wakefulness, the EEG revealed

decreased β-activity in IH patients when compared to controls in all waking EEG recordings (i.e., across all time points). EEG β-activity is hypothesized to reflect brain arousal, attention, alertness, and reactivity. Decreased β-activity could indicate insufficient arousal levels and provide a physiological measure of daytime sleepiness in IH patients. During the 40 hours awake, patients with IH rated themselves as sleepier than controls, and the largest difference was seen within the first 16h awake. Neurobehavioral performance data revealed a similar picture. Patients performed worse during the sleep deprivation period than controls with a maximal deviation during the first 16h awake.

In conclusion, we studied for the first time a role for mGluR5 in relation to sleep and extended wakefulness. We observed increased mGluR5 availability after prolonged wakefulness, which correlated positively with sleepiness. These results may indicate that this receptor could present a potential drug target for the treatement of excessevie sleepiness. The second project was the first sleep deprivation study performed in IH patients. It revealed normal sleep-wake regulation in IH patients, yet a deficient brain arousal system during wakefulness. This finding provides a rational basis for clinicians to study more extensively daytime functions in patients with IH in the future.

Zusammenfassung

Schlaf ist ein allgegenwärtiges Phänomen unter den Tieren. Die Charakteristiken des Schlaf- und Wachverhaltens können je nach Art sehr unterschiedlich sein. Das Schlafverhalten reicht von nur einem Auge geschlossen halten (Ente), nur eine Hirnhälfte im Schlafzustand haben (Delphin), Apnöen in Atemmuster aufweisen (Robbe), ein nachtaktives Verhalten ausüben (Fledermaus) oder einen polyphasischen Schlafrhythmus aufweisen (Maus) bis hin zum monophasischen Schlafmuster des Menschen. Obwohl vieles über die Funktion des Schlafes noch verborgen ist, wissen wir, dass der Schlaf eine wichtige Funktion erfüllt. Er hat die Evolution überstanden und scheint demzufolge eine lebenswichtige Funktion auszuüben.

Diese Arbeit erforscht die Schlaf-Wachregulation beim Menschen. Den Schlaf zu untersuchen ist von hohem Interesse, weil wir die Charakteristiken dieses Zustandes nicht bewusst beeinflussen können. Schlaf ist ein Zustand mit vermindertem Bewusstsein und reduzierter Reaktionsfähigkeit.

Es ist interessant, die Komplexität des Schlafes zu untersuchen. Schlafstadien, die Schlafarchitektur, der Zeitpunkt des Schlafs, die Schlafdauer und die Schlafhomöostase sind nur einige Aspekte, die studiert werden können. Die unterschiedlichen Merkmale der Hirnwellen und deren Generierung im Gehirn während des Schlaf- und Wachzustandes sind von grosser Wichtigkeit in der Schlafforschung.

Die δ-Aktivität ist ein Merkmal des Schlaf-Elektroenzephalograms, auf welcher das bekannte und weitherum akzeptierte Zwei-Prozess Modell der Schlaf-Wachregulation beruht. Dieses Modell besagt, dass der Zeitpunkt und die Dauer des Schlafs und der Wachheit das Resultat zweier interagierender Prozesse darstellen. Der homeostatische Prozess ist von der Dauer der vorangegangenen Wachzeit abhängig. Dagegen ist der zirkadiane Prozess endogen reguliert, über

die suprachiasmatischen Kerne im Hypothalamus. Das Modell der synaptischen Homöostase liefert eine mögliche zelluläre Erklärung des homöostatischen Prozesseses und diente zusätzlich als theoretische Basis für meine Arbeit.

Das **Ziel meiner Arbeit** war, einen möglichen Mechanismus des homöostatischen Prozesses auf molekularer Ebene zu untersuchen. Dazu studierten wir die Rolle des metabotropen Glutamat-Rezeptors des Subtyps 5 (mGlu5 Rezeptoren) bei gesunden Probanden. Desweitern habe ich den homöostatischen Prozess auf einer physiologischen Ebene bei Patienten mit idiopathischer Hypersomnie studiert. Wir haben den homöostatisch regulierten Schlafdruck bei gesunden und kranken Probanden durch die Verlängerung der Wachzeit bis 40 Stunden experimentell erhöht.

Das **erste Projekt** meiner Arbeit (Kapitel 2) befasst sich mit dem Effekt von Schlafentzug auf die Ausprägung der mGlu5 Rezeptoren bei gesunden männlichen Versuchspersonen und einer möglichen Assoziation mit kognitiven Funktionen. Diese Positron Emissions Tomopgrahie (PET) Studie wurde mit dem hoch selektiven Radioliganden ^{11}C-ABP688 in einem randomisierten und überkreuzten Ablauf durchgeführt. Zweiundzwanzig freiwillige Männer haben das Studienprotokoll vollständig durchlaufen. In der Kontrollbedingung wurde die PET Messung nach 9 Stunden Wachzeit durchgeführt. In der Schlafentzugsbedingung wurde die PET Messung nach 32 Stunden Wachzeit, beziehungsweise nach einer Nacht ohne Schlaf, durchgeführt. Die Studie hat gezeigt, dass eine Nacht ohne Schlaf die mGlu5 Rezeptor-Verfügbarkeit im Gehirn global ansteigen lässt. In 6 vorgängig festgelegten Regionen (regions of interest), welchen in früheren Studien wichtige Rollen bei der Schlaf-Wachregulation zugeschrieben wurden, konnte eine Erhöhung gemessen werden. Die Zunahme korrelierte positiv mit der Zunahme der subjektiven Schläfrigkeit nach Schlafentzug.

Das **zweite Projekt** meiner Arbeit (Kapitel 3) versuchte ein besseres Verständnis der Pathophysiologie der idiopathischen Hypersomnie (IH) zu erlangen. Indem wir Patienten mit IH 40 Stunden wach hielten, konnten wir Aspekte der homöostatischen und zirkadianen Prozesse untersuchen. Diese Studie wurde in einer gepaarten Anordnung mit gesunden Kontrollpersonen durchgeführt. Die visuell definierten Schlafstadien unterschieden sich weder in der Baselinenacht noch in der Erholungsnacht zwischen den beiden Gruppen. Des weiteren wurde in beiden Gruppen im non-rapid eye movement Schlaf (NREMS) ein ähnlicher Verlauf des Abfalls der δ-Aktivität beobachtet. Dennoch haben wir im EEG einen Unterschied im Bereich der θ-/α-, σ- und β-Frequenzen festgestellt. Patienten mit IH hatten im NREMS und REMS eine erniedrigte Leistungsdichte im Vergleich zu den gesunden Kontrollen. Auch während der verlängerten Wachzeit, zeigten alle Wach-EEG Messungen im Vergleich zu den Kontrollen bei den Patienten mit IH eine reduzierte β-Aktivität. Es wird vermutet, dass die β-Aktivität im EEG den Wachheitszustand des Gehirns, Aufmerksamkeit, Wachheit und Reaktionsbereitschaft widerspiegelt. Die reduzierte β-Aktivität könnte den zu geringen Wachheitszustand des Gehirns anzeigen, und ein physiologisches Merkmal der erhöhten Tagesschläfrigkeit darstellen. Während den 40 Stunden Wachheit, haben sich die Patienten schläfriger eingeschätzt als die gesunden Kontrollen. Die grösste Differenz konnte während den ersten 16 Stunden beobachtet werden. Die Daten der anhaltenden Aufmerksamkeit zeigten ein ähnliches Bild. Die Resultate eines validierten Reaktionstests zeigten während der ganzen Wachzeit eine schlechtere Leistung bei den Patienten im Vergleich zu den Kontrollen mit einer maximalen Divergenz während den ersten 16 Stunden nach dem Erwachen.

Meine Arbeit untersuchte zum ersten Mal einen möglichen Zusammenhang zwischen der Verfügbarkeit von mGlu5 Rezeptoren und Schlafentzug. Wir haben nach verlängerter Wachzeit eine klare Erhöhung der Rezeptorverfügbarkeit festgestellt, welche mit der subjektiven Schläfrigkeit positiv korrelierte. Dieser Befund könnte auf eine mögliche neue Zielstruktur für die pharmakologischen

Behandlung von erhöhter Tagesschläfrigkeit hindeuten. Das zweite Projekt war die erste Schlafentzugsstudie bei Patienten mit IH. Sie zeigte eine normale Schlafregulation; dagegen war der Wachheitszustand des Gehirns am Tag reduziert. Dieses Resultat legt nahe, sich in Zukunft mehr auf das Studium der Wachfunktionen bei Patienten mit IH zu verlegen.

Abbreviations

^{11}C-ABP688	3-(6-Methyl-pyridin-2-ylethynyl)-cyclohex-2-enone-O-11C-methyl-oxime
$A_{2A}R$	Adenosine A_{2A} receptor
AAS	Ascending arousal system
ACC	Anterior cingulate cortex
Ach	Acetylcholine
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BDNF	Brain-derived neurotrophic factor
B/I	Bolus-infusion
BL	Baseline night
BF	Basal forebrain
BP	Binding Potential
Ca	Calcium
CNS	Central nervous system
D_2	D2 Dopamin receptor
DV	Distribution volume
DVnorm	Normalized distribution volume
ECG	Electrocardiogram
EDS	Excessive daytime sleepiness
EEG	Electroencephalogram
EMG	Electromyogram
EOG	Electrooculogram
ESS	Epworth sleepiness scale
FFT	Fast fourier transformation
FMR1	Fragile mental retardation protein 1
fMRI	Functional magnetic resonance imaging
FXS	Fragile X syndrome
GABA	γ-aminobutyric acid
GLU	Glutamate

GluR	Glutamate receptor
Gy	Gray (Unit)
Hcrt / Orx	Hypocretin / Orexin
ICSD-2	International criteria of sleep disorder 2nd edition
IH	Idiopathic hypersomnia
IP3	Inositol triphospaht
KSS	Karolinska sleepiness scale
LC	Locus coeruleus
LDT	Laterodorsal tegmental nuclei
LHA	Lateral hypothalamus
LTD	Long-term depression
LTP	Long-term potentiation
Mg	Magnesium
mGluR	Metabotropic glutamate receptor
mGluR1/5	Metabotropic glutamate receptor subtype 1/5
MRI	Magnet resonance imaging
MT	Movement time
µV	microvolt
NA	Noradrenalin
NC	Narcolepsy
NMDA	N-methyl-D-aspartate
NREM	Non-rapid-eye-movement
PET	Positron emission tomography
PFC	Prefrontal cortex
PLC	Phospolipase C
PLMS	Periodic leg movement of sleep
PK	Protein kinase
PMOD	Pixel-wise image modeling
POMS	Profile of mood state
PPT	Pedunculopontine tegmental nucleus
Process C	Circadian process of the two process model

Process S	Homeostatic process of the two process model
PSG	Polysomnography
PVT	Psychomotor vigilance task
REC	Recovery night
REM	Rapid-eye-movement
RF	Reticular formatio
RL	REM latency
RT	Reaction time
S1 - 4	Sleep stage 1 - 4
SD	Sleep deprivation
SCN	Suprachiasmatic nucleus
SL	Sleep latency
SOREMS	Sleep onset REM sleep
STAI	State trait anxiety inventory
SUV	Standard uptake value
SWA	Slow-wave activity
SWS	Slow wave sleep
TAC	Time activity curve
TMN	Tuberomammillar nucleus
TPQ	Tridimensional personality questionnaire
TST	Total sleep time
VLPO	Ventrolateral preoptic nucleus
VOI	Volume of interest
W	Wake
WASO	Wake after sleep onset

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Chapter 1

Introduction

1.1 The brain as a bioelectric generator

The human brain is a complex organ where thousands of interacting processes occur at the same time. The signals coming from the brain influence different aspects of human metabolism and physiology, one of which is sleep-wake regulation. The brain can be seen, beside its many other functions, as a bioelectric generator. Whereas an electric generator is transforming kinetic energy into electrical energy, the brain is transferring chemical cell currents into cell dipoles, which lead to an electrical field. These cortical electrical fields can be measured with the non-invasive method of electroencephalography (EEG). The inventor of this method was the German psychiatrist Hans Berger from Jena who accomplished the first human recordings, typically on his children, in the 1920's (Berger, 1929). To measure the EEG, at least two surface electrodes are needed which record the potential difference between them. One is the reference electrode, and the other is the electrode where the local signal is measured. The usage of several electrodes makes it possible to study topographic aspects of brain waves. The correct placement of the electrodes is defined in the 10-20 system, invented 50 years ago by Herbert Jasper (Jasper, 1958). Electrodes are placed at well-defined inter-electrode distances of 10% and 20% over frontal, parietal, occipital, and temporal regions of the scalp (Fig. 1).

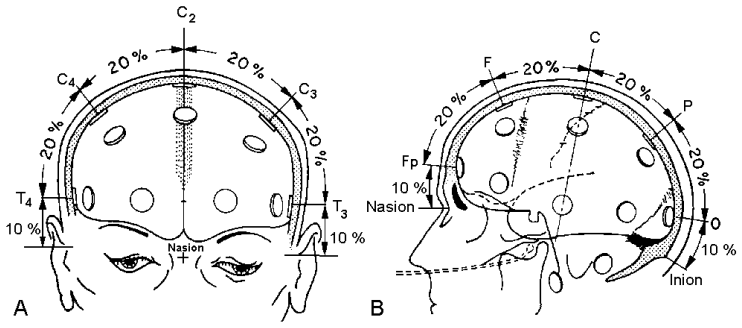

Figure 1: The original figure illustrates the international 10-20 system (Jasper 1958).
Please note that the electrodes are erroneously located inside the skull on the surface of the cortex.

Three vigilance states can be distinguished in human beings: Wakefulness, non-rapid-eye-movement sleep (NREMS), and rapid-eye-movement sleep (REMS). In the wake state, when a person is fully conscious, the rather high frequency α-activity is dominant, and towards deep sleep the low frequency and high amplitude δ-activity becomes prominent. NREMS and REMS are conditions of a reduced state of consciousness and cannot be influenced voluntarily by a sleeping person.

The waking electroencephalogram

It is assumed that the rhythmic activity measured in the EEG represents cellular feedback loops between deep brain structures (e.g., thalamus) and cortical regions. The better these feedback loops become integrated and interconnected with other brain areas, the faster the frequency of EEG oscillations will be (Klimesch, 1999). Each brain wave can be decomposed into distinct amplitudes and frequencies which are assigned to different frequency bands. The most commonly used nomenclature in wakefulness is δ, θ, α, β and γ (Niedermeyer, 2005). The frequency bands definition in wakefulness varies between studies. One study specified these bands with the frequencies < 3.5 Hertz (Hz) as δ, 4-7.5 Hz as θ, 8-13 Hz as α, 14-30 Hz as β, and > 30 Hz as γ (Niedermeyer, 2005). The most prominent rhythmic activity during relaxed wakefulness is the α-wave. When a person closes his/her eyes, the amplitude of this wave becomes

larger and is seen best in occipital regions. A hallmark of the α-activity is its dropout with the earliest approach of drowsiness. From a topographical point of view, faster rhythms are present in posterior regions, whereas the slower waves are more prominent on anterior sides. Age is suggested to play a role in the modulation of the α-activity. The acceleration is thought to end around the age of ten, and tends to show little or no decline in elderly subjects. This decline is a result of age related neurological diseases, or even a lack of mental training in later life, rather than an effect of age per se (Klimesch, 1999; Niedermeyer, 2005). Different studies show a relation between α-activity and cognitive performance, reaction time, intelligence, or stress and anxiety. Even though the amplitudes vary considerably from individual to individual and, within a person, from moment to moment, there is evidence that body temperature, menstrual cycle and genetic background of a person modulate the shape of the α-frequency (Bodenmann et al., 2009b). A twin study reports a high heritability of 89% and states the rhythmic brain-electrical activity as one of the most heritable characteristics in humans (van Beijsterveldt et al., 1996). It can be assumed that there are cortico-cortical and thalamo-cortical systems which interact in the generation of cortical α-rhythms (Steriade et al., 1990). In addition, the β-activity is thought to represent an important physiological marker. The β-rhythm was discovered by Berger in 1930, and is most prominent over frontal and central regions. Beta-activity seldomly exceeds an amplitude of 30 μV (Klimesch, 1999; Niedermeyer, 2005). A gender difference was found by Matsuura, suggesting that females have more prominent β-activity than males (Matsuura et al., 1985). Walter and Dovey (Walter and Dovey, 1944) introduced the θ-activity which is better seen in children than in adults (Klimesch, 1999; Niedermeyer, 2005). In animals the θ-rhythm represents a prominent sinusoidale waveform generated in the hippocampus and has wide frequency range (3-12 Hz) and high power. A crucial finding is that with increasing cognitive demands, especially in memory tasks, short-term memory demands lead to θ synchronization (increase in band power), whereas long-term memory demands lead to task specific desynchronization (decrease in power) in the upper α band (Klimesch, 1996).

Increased sleepiness was referred to increased θ-activity and lower α-activity (Cajochen et al., 1995; Aeschbach et al., 1997). Furthermore, hippocampal θ-activity is thought to be important in the induction of long-term potentiation (LTP), and is suggested to be related to the encoding of new information in a similar way to LTP (Kamondi et al., 1998; Tsanov and Manahan-Vaughan, 2009).

Brain waves during sleep and their classification

Sleep is neither a passive nor a continuous state. Sleep is an active condition with region and network specific brain activity. Sleep is a cyclic alternation between NREMS and REMS (Aserinsky and Kleitman, 1953; Dement and Kleitman, 1957; Borbély and Achermann, 2005). Nocturnal sleep can be roughly divided into four to five consecutive NREMS/REMS cycles with durations of approximately 90 to 120 minutes (Feinberg and Floyd, 1979).

The sleep EEG can be quantified by the differentiation of the brain waves into amplitude and frequency. This information, together with the electroocculogram (EOG) and electromyogram (EMG) signals, is used to assign the brain waves into specific sleep stages. In the late 1960's, the manual of standardized terminology was published by Rechtschaffen and Kales (Rechtschaffen and Kales, 1968). They distinguish between six states: Wakefulness (W), Movement time (MT), Stages 1 to 4 (S1-S4), and REMS. NREMS is the sum of epochs scored with S1, S2, S3 and S4. The characterization of wakefulness is, as already mentioned, the dominant α-activity and/or mixed frequency activity and low power (Fig. 2A). Movement time occurs when a person changes his/her position in bed. The EMG signal is recorded by placing two electrodes beneath the chin. This raw signal shows high amplitude activity, and the EEG and EOG signals show, as a result, movement artifacts. Muscle twitches often occur in S1 while falling asleep. Nocturnal sleep is normally initiated by S1, which is the transient state between wakefulness and sleep. When a subject falls asleep, rolling eye movements occur and the EEG typically shows θ-waves with mixed-frequency activity and low power. Stage 2 indicates two distinct features called sleep spindles (11-15 Hz) and K-complexes. A sleep spindle is defined by

duration of at least 0.5 seconds. The shape is given by waxing and waning amplitudes of 6 to 7 distinct waves within one spindle. K-complexes are shaped by a negative half wave followed by an even bigger positive half wave (Fig. 2B). The amount of S2 epochs is increasing towards the end of the night, and is the most frequently occurring sleep state with around 55% of total sleep time. Stages 3 and 4 are often taken together and are referred to as deep sleep, or slow-wave sleep (SWS). The physiological correlates are the slow oscillation with frequencies < 2 Hz and peak-to-peak amplitude > 75 μV (Fig. 2C). A discrete change in EOG and EMG signals is seen from the transition of NREMS to REMS. REMS was identified and defined by Eugene Aserinsky and Nathaniel Kleitman in the early 1950's (Aserinsky and Kleitman, 1953). The eye movements occur in all directions and are binocularly synchronous (Dement and Kleitman, 1957). The EMG signal shows a gradual diminution as sleep approaches, and is essential to clearly assign the REMS stage. A typical feature, even though not shown by all subjects, is the muscle twitch shown by a single peak in the EMG signal. For a long time, it was believed that REMS is the dreaming phase. But in 1957, Dement and Kleitman (Dement and Kleitman, 1957) suggested, and partially clarified, that dreaming can occur in all sleep stages and is not restricted to REMS. The behavioral features of REMS are low muscle tone, also referred to as muscle atonia, and is controlled by glutamatergic and glycinergic projections into the spinal cord (Siegel, 2004), and saccadic eye movements (Fig. 2D). The EEG frequency and amplitude are similar to S1 with the exception that no vertex sharp waves occur, and sleep spindles and K-complexes are absent. While at sleep onset in the evening (when entrained), the propensity for NREMS is high and for REMS low, the propensities are practically equal by the end of the sleep period. Furthermore, REMS modulates physiological markers such as heart rate or blood pressure (Somers et al., 1993). In contrast to deep slow-wave sleep (S3+S4; SWS), REMS becomes more prominent in the second half of the night.

As illustrated above, sleep reflects a cycling alternation between NREMS and REMS. Sleep architecture can be visualized by a hypnogram. A hypnogram (Fig.

3) is generated by assigning one sleep stage to each 20- or 30-second epoch of the night. An epoch is a short time window, usually lasting 20 or 30 seconds, of recorded brain activity. A typical night of sleep (e.g., 8 hours) will be split up into 1440 epochs which need to be manually scored. Even though this scoring procedure is based on arbitrary criteria, it was shown to have high interrater reliability (Danker-Hopfe et al., 2009). Nevertheless, a better and more accurate approach was needed to quantitatively analyze the sleep EEG. This was achieved by calculating the power spectrum of the night (Section 1.2). The slow-wave activity (SWA) 0.5-4.5 Hz is a correlate of SWS, and is referred to as a marker of sleep propensity; it therefore reflects the homeostatic process S (see Section 1.3) (Borbély, 1982; Cajochen et al., 1999; Finelli et al., 2001b; Borbély and Achermann, 2005) and shows a dynamic decline with respect to time spent asleep (Fig. 3).

In the last few years, several studies indicated that the EEG signal is 'trait-like' and can be seen as an individual physiological fingerprint. A study in monozygotic (MZ) and dizygotic twins (DZ) showed a high heritability in the δ-range and the spindles (Ambrosius et al., 2008). Monozygotic twins showed a higher within-pair resemblance in spectral power than DZ, which reflects the high heritability of this phenotype. Another twin study showed a heritability in the 8-16 Hz range of 96% (De Gennaro et al., 2008); Finelli showed that even the topographical feature in the EEG shows a fingerprint-like manner and may reflect individual traits of functional anatomy (Finelli et al., 2001a).

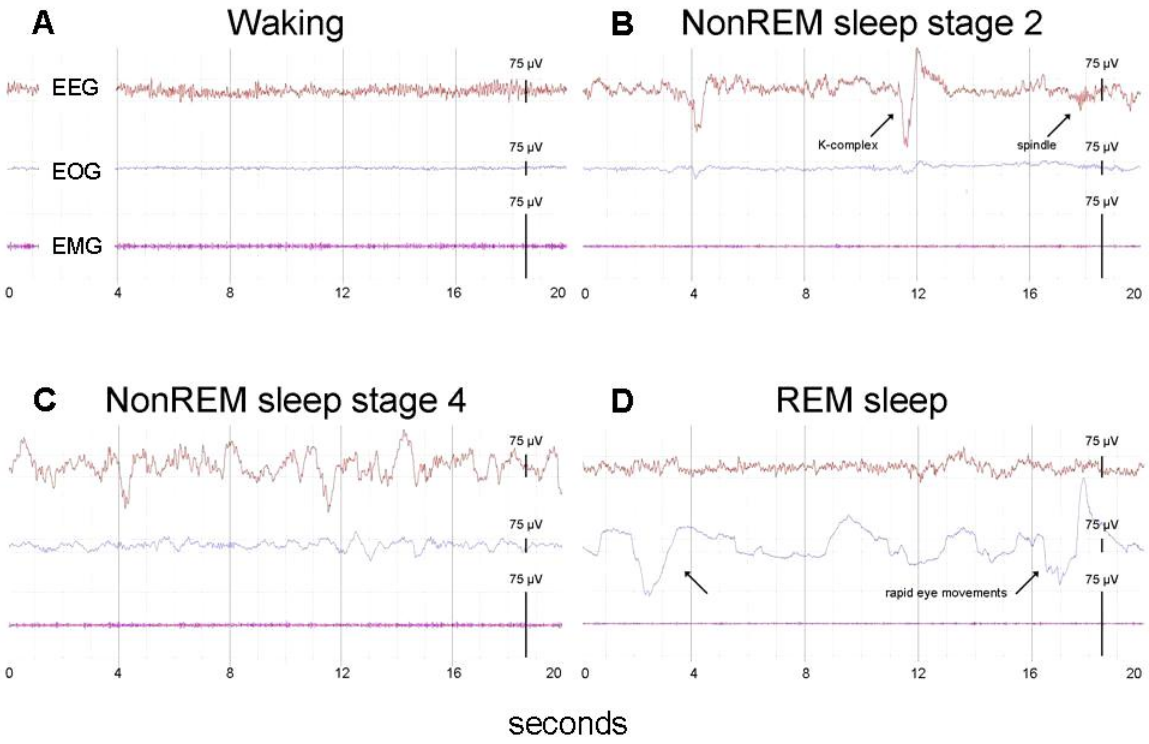

Figure 2: Electrical raw signal in the three vigilance states. **A** The waking EEG typically shows mixed frequency activity and low amplitude brain waves with a prominent occurrance of α-waves (8-12 Hz). Eye blinks are indicated by a spike in the EOG. The muscle tone is rather high. **B** Stage 2, NREM sleep, has two recurrent features named the K-complexes and the sleep spindles (11-15 Hz) on a mixed frequency and low-voltage background. **C** Stage 4, NREMS, refers to deep sleep with the hallmark of low frequency (0.5-4.5 Hz), high amplitude slow waves. The EMG is low, reflecting a physiological relaxed state. **D** REMS, shows the distinct properties of random saccadic eye movement and a very low muscle tone reflecting muscle atonia. EEG signal shows mixed frequency, low voltage and amplitude.

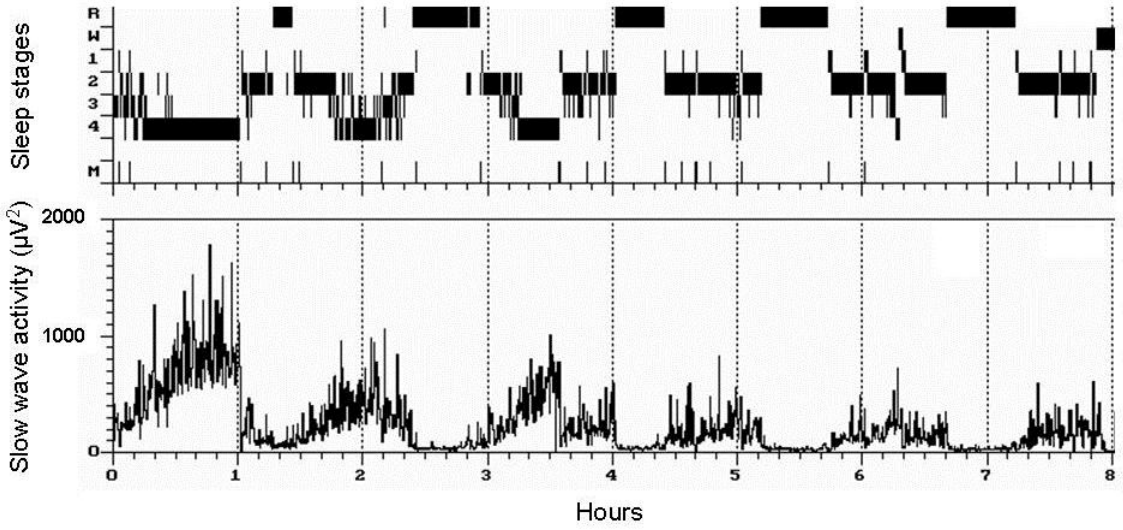

Figure 3: Hypnogram and slow-wave activity in a healthy subject. **Upper panel:** Manually scored sleep stages during an 8-hour sleep opportunity. R: REMS, W: Wake, 1-4: Sleep stage 1-4, M: Movement time. **Lower panel:** The corresponding slow-wave activity (0.5-4.5 Hz) shows a decreasing trend across the night.

1.2 The quantification of the electroencephalogram

In a physiological sense, EEG power reflects the number of neurons that discharge synchronously. The raw signal is made up of two variable entities: The amplitude (microvolt/μV), and the frequency (Hz) which is displayed against time. The amplitude of the EEG, when measured with depth brain electrodes, lies between 500-1500 μV, and attenuates the further away from the signal generating cells the recording takes place. The amplitude of the EEG is about 10-100 μV when measured on the scalp (Aurlien et al., 2004).

Brain activity is recorded with a specific frequency rate; it is filtered, digitized and transmitted to a computer where the signal is stored.

Artefacts are visually identified and excluded before the raw signal undergoes a Fast Fourier transformation (FFT) procedure. The FFT is a mathematical approach to quantify the EEG raw signal. Most often, the bipolar derivation C3A2

is used for this procedure (Cooley and Tukey, 1965). If topographical analyses are performed, additional derivations undergo FFT. An all-night power spectrum is generated by the FFT for each 20-seconds time window in MATLAB (The MathWorks, Natick, MA). The principle of the FFT is a decomposition of the raw signal into different amplitudes and frequencies per time window. The time window is short (4s in sleep, 2s in wake), in order to have a stationary signal which is a prerequisite of FFT (Achermann, 2009). The resolution is the inverse of the length of the analysed epoch (0.25 Hz in sleep, 0.5 Hz in wake). In short, the algorithm transforms the digitised EEG signal from the time domain into the frequency domain. In Figure 4, the characteristic power spectrum of NREMS, REMS and wakefulness are illustrated.

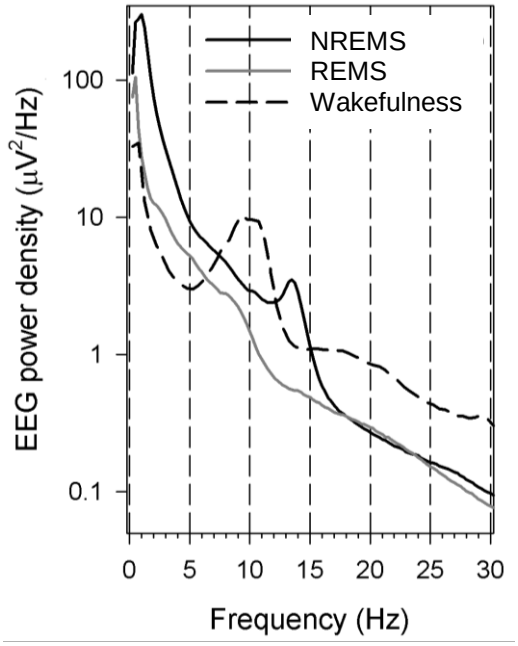

Figure 4. Spectral characteristics of the EEG (C3A2 derivation) in NREMS, REMS and rested wakefulness. NREMS is characterised by dominant rhythmic activity in low frequencies resulting in high power, and a second peak in the σ-range. REMS, in contrast, has a gradual decrease in power with increasing frequency. Wakefulness has highest power < 1 Hz and a second maximum in the α-range. Moreover, β-power is higher in wakefulness compared to NREMS and REMS.
NREMS (combined stages 1 to 4) and REMS: All-night power spectra of an 8-hour baseline night (00:00 – 08:00) in 22 healthy men (mean age ± SEM: 23.4 ± 0.5 years). Frequency resolution: 0.25 Hz. Wakefulness: Average over four 3-min EEG recordings with closed eyes conducted at 08:15, 11:00, 14:00 and 17:00 following the baseline night. Frequency resolution: 0.5 Hz. Data taken from the study performed by (Bodenmann et al., 2009a).

1.3 The two process model of sleep wake regulation

The established, widely accepted two-process model of sleep wake regulation (Fig.5) was postulated by the internationally well-known sleep researcher Alexander Borbély in 1982. It posits that the interaction of the homeostatic process (S) and the circadian process (C) generates the timing of sleep and waking (Borbély, 1982). The homeostatic process increases during wakefulness and declines during sleep. The process C is guided endogenously via the suprachiasmatic nucleus (SCN) in the hypothalamus. The pacemaker with only 20000 neurons displays a periodicity of approximately 24 hours (Moore and Eichler, 1972; Stephan and Zucker, 1972), and modulates an upper and lower threshold (H and L) (Fig.6). The timing and termination of a sleep episode is, on the one hand, defined by these two thresholds and, on the other hand, by the intersection of curves S and C (Borbély, 1982; Achermann, 2004).

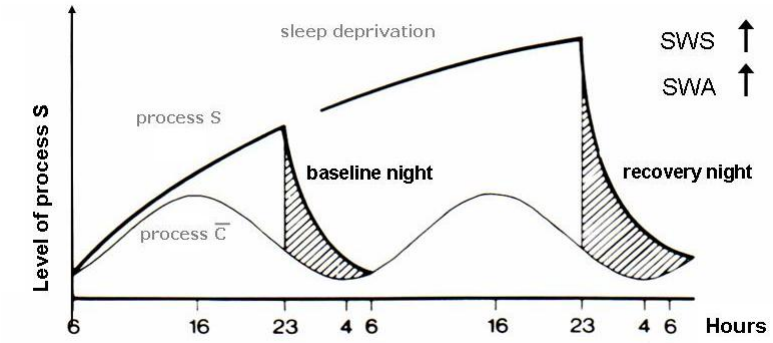

Figure 5: Two-process model of sleep wake regulation. The interaction of process S and C determines the waking and sleep periods. After prolonged wakefulness the SWA will be enhanced in the subsequent recovery night compared to baseline night (Borbély, 1982). SWS: Slow wave sleep; SWA: Slow-wave activity; Process S: Homeostatic process; Process C: Circadian process.

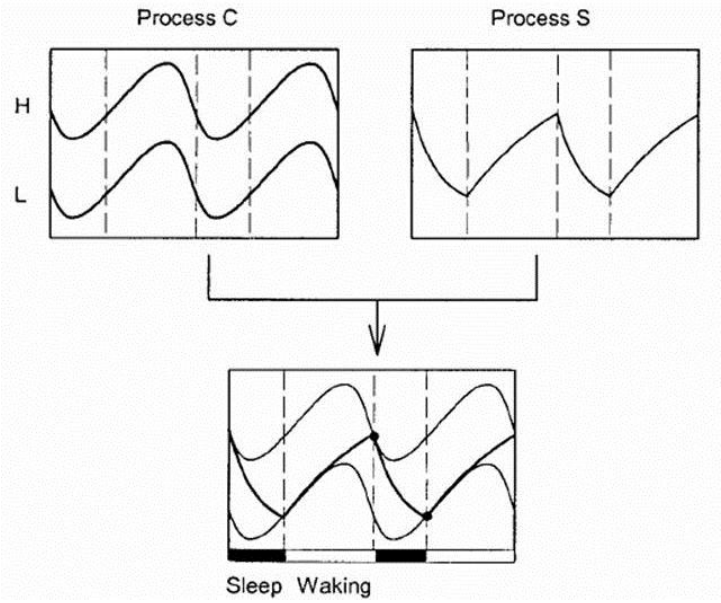

Figure 6: Schematic representation of the two-process model of sleep regulation. Process S rises during waking and declines during sleep. Process C modulates the thresholds of H and L. Interaction of Process S with H and L determines the onset and termination of a sleep episode (Achermann, 2004). Process S: Homeostatic process; Process C: Circadian process, H: high threshold of circadian process; L: low threshold of circadiane process.

It needs to be mentioned that sleep has an intensity dimension which is increased after prolonged wakefulness. The other characteristic of sleep is sleep duration, which also increases after prolonged hours awake (Borbély, 1982; Daan et al., 1984).

Sleep intensity corresponds to the summation of S and C, and is therefore represented by the difference between S and C (Fig.5). The physiological marker of process S during the biological night is derived from the SWA, a correlate of the δ-activity, which shows an exponential decay during the night and may represent an intensity parameter of the sleep process. A possible physiological correlate of homeostasis during wakefulness is thought to be reflected by the power in the θ-/low α-frequency range. It was shown that increasing sleep need during prolonged hours awake leads to an enhancement in θ-/low α-band in the waking EEG (Cajochen et al., 1995; Aeschbach et al., 1997; Finelli et al., 2001b). It is assumed that the increase of θ-activity in the waking EEG during extended

wakefulness is closely related to the enhancement of SWA in recovery NREMS (Finelli et al., 2000).

Mathematical modelling helps to delineate the processes involved in the regulation of sleep and neurobehavioral function, and offers thereby a conceptual framework for the analysis of existing and new data. Mathematical functions were fit to available data pools to estimate the build up of process S during wakefulness and its decay during sleep. It was hypothesized that process S is increasing according to an exponential saturating function during wakefulness, and is declining roughly exponentially during sleep (Achermann, 2004). It was first quantified on the basis of baseline data and recovery sleep after sleep deprivation (Daan et al., 1984). Further investigations proposed a non-linearity of the interaction of the two processes to predict wakefulness and neurobehavioral performance during the day, and to promote consolidated sleep throughout the night (Van Dongen et al., 2003). A robust method was established by Rusterholz et al. (Rusterholz et al., 2010) to estimate the process S on an individual basis. Process S was modelled for each person by a saturating exponential function during wakefulness and an exponential decline during sleep.

Sleep deprivation (SD) is a useful tool to challenge and study sleep homeostasis. Partial or total SD leads to an increase in SWA in the subsequent night. Compelling evidence was presented in several studies that show how SWS and SWA increase as a function of prior hours spent awake (Webb and Agnew, 1971; Achermann and Borbély, 2003; Achermann, 2004). In addition, it has been shown that frontal regions are particularly sensitive to SD, thus leading to impairments in cognitive functions (Horne, 1993; Huber et al., 2000).

Even though it is known that SWS and REMS have opposite trends in nighttime sleep, the results of sleep deprivation studies were rather surprising. As expected, SWS was enhanced in recovery sleep, whereas REMS was not altered, or in some instances even decreased. The rebound of SWS was mainly seen in the first recovery night (REC) by an increased δ-power (Agnew et al.,

1967). In REMS deprivation studies, an increased REMS proportion, which was present for more than one night, was found in a recovery night (Dement, 1960; Dement, 1965; Moses et al., 1975).

Another aid to explore and modulate process S is a nap protocol study. Naps are a useful tool to study the level of slow wave propensity after various durations of waking. Naps taken later in the day contain more SWS than naps taken earlier in the day (Maron et al., 1964; Achermann and Borbély, 2003).

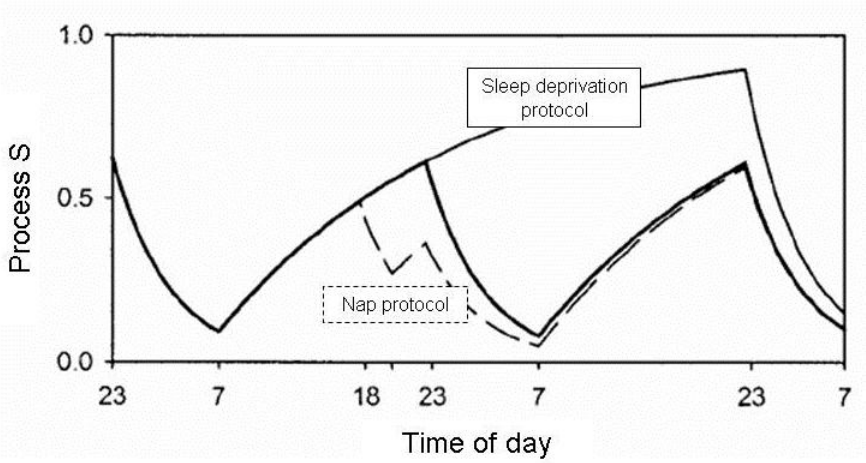

Figure 7: Schematic representation of the evolution of process S during 2-hour-nap and total-sleep-deprivation protocols (Achermann, 2004).

Not only do manipulations of time spent awake, or sleeping, lead to a change in process S, but genetic factors may also contribute to individual differences in vigilance states and therefore in sleep homeostasis (Rétey et al., 2005; Viola et al., 2007; Landolt, 2008a).

Recently, a study has shown that the prominent occurrence or attenuation of distinct brain waves during sleep may reflect cerebral activity during wakefulness prior to sleep. For example, authors observed that sleep SWA reflecting synchroneous neuronal firing, was reduced over the sensorimotor cortex contra-lateral to the arm which was immobilized during the day. These findings point to local aspects of sleep and sleep regulation (Huber et al., 2004; Huber et al.,

2006). A study performed in neurosurgical patients showed similar findings (Nir et al., 2011). The simultaneous recording of scalp EEG, intra cerebral EEG, and unit firing showed that most sleep slow waves (< 1 Hz) and the underlying active and inactive neuronal states occur locally (Nir et al., 2011). It demonstrates that some brain areas are active while others are silent. A further study suggested that local sleep also occurs in awake rats. Neuronal 'off' periods are features occurring in sleep. The study showed that cortical neurons in rats, which were awake for long periods, can go locally 'offline', as detected by slow-waves in the EEG (Vyazovskiy et al., 2011). Although the exact mechanisms remain to be established, a considerable number of reports indicate that local activation of brain regions during wakefulness results in sleep EEG changes in these regions. This supports the hypothesis that local aspects of sleep regulation may reflect recovery or reactivation processes in brain areas that have been used during wakefulness (Benington and Heller, 1995).

1.4 Physiological and cellular properties of synaptic plasticity

"When an axon of cell A… excites cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B is increased."

(Donald Olding Hebb, 1949)

Out of the prominent quotation of Donald O. Hebb the area of synaptic plasticity emerged. In short: Simultaneous activation of interconnected cells leads to modification of synaptic components and changes in gene expression, and is followed by pronounced increases in synaptic strength which is referred to as functional plasticity.

A further very well known statement by Hebb summarises the whole theory in just a few words.

"Cells that fire together, wire together"

Donald Olding Hebb

1904 - 1985

General overview

Charles Scott Sherrington was the person who named the site of action which Donald O. Hebb described as 'a synapse'. Synaptic plasticity is the ability of the connections between two neurons to change in strength in response to either use or disuse of transmission over synaptic pathways. Neuroscientists refer to the 'disuse' as long-term depression (LTD), and to the 'use' as long-term potentiation (LTP). Long-term potentiation and LTD appear to be essential in the stabilization and elimination of synapses during the developmental fine-tuning of neural circuits and, therefore, lead to behavioural changes. One consequence of high interest is the belief that LTP is an underlying mechanism for learning and memory in vertebrates.

Long-term potentiation was first described in 1973 by Tim Bliss and Terje Lømo (Bliss and Gardner-Medwin, 1973b, 1973a; Bliss and Lomo, 1973), and has been, since then, of more interest than LTD. Bliss and Lømo found in rabbit hippocampus that a few seconds of high frequency electrical stimulation can enhance synaptic transmission for days, or even weeks.

The primary model of LTP investigation is the hippocampus, which is an anatomical structure strongly involved in learning. The best understood form of LTP is induced by the activation of the N-methyl-d-aspartate (NMDA) receptor complex. This receptor allows electrical events at the postsynaptic membrane to be transducted into chemical signals, which are thought to activate both pre- and post-synaptic mechanisms to generate a persistent increase in synaptic strength (Bliss and Collingridge, 1993). NMDA receptors (NMDAR) are referred to as a coincidence detector, because of the suggested ability to be able to respond to

the conjunction of activity in afferent fibres and adequate depolarization in target dendrites.

Possible biochemical mechanisms

Synaptic plasticity occurs when changes in gene expression and postsynaptic density complex take place at the same time. It needs the signalling of biochemical neurotransmitters to induce protein synthesis or spatial reorganisation.

Long-term plasticity includes LTD and LTP, which have opposite effects regarding the strengthening or weakening of signaling between two neurons.

Long-term depression

LTD can be either activity-dependent or independent. What remains the same is the selective weakening of specific synaptic strength and, further, their efficacy in many areas of the brain. In simple words, LTD occurs when the postsynapse is not stimulated enough to induce LTP. Weak activation of NMDAR leads to a modest membrane depolarization, and to only a small rise in postsynaptic calcium (Ca^{2+}) concentration. The lower concentration of Ca^{2+} leads to an activation of intracellular signaling molecules different from those in LTP, including serine phosphatases, which dephosphorylate critical synaptic substances, including the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid Receptor (AMPAR) (Malenka and Bear, 2004).

Long-term potentiation

In contrast, LTP aims to optimize the signaling between two neurons. The three basic characteristics are: Cooperativity, associativity and input-specificity (Bliss and Collingridge, 1993). The molecular mechanism of LTP is most often induced by the activation of NMDAR at the post-synapse. AMPA channel is a receptor which modulates the action of the colocalized NMDAR (Siegel et al., 1995; Kharazia et al., 1996). The opening of NMDA channels is dependant on the degree of depolarization, and leads to a rise in Ca^{2+} concentration and an

activation of protein kinases (PK) in the post-synapse. A specific property of the NMDAR channel protein is the voltage-dependent blockage by magnesium (Mg^{2+}). Only strong depolarization of the post-synaptic cell can displace Mg^{2+} from the channel, allowing calcium ions to enter. The increasing intracellular calcium concentration further leads to an increased excretion of intracellularly stored calcium (Ca^{2+}) which activates downstream pathways, for example, the prominent change in AMPAR trafficking that results in an increased number of AMPAR in the post-synaptic plasma membrane with no effect on NMDAR. It is accompanied by observed enlargement of dendritic spines and associated postsynaptic densities (Yuste and Bonhoeffer, 2001; Malenka and Bear, 2004).

There is evidence that NMDAR itself is not sufficient to induce LTP. Elements such as metabotropic glutamate receptor (mGluR) may be needed to induce long lasting cellular changes. MGluR-dependant LTP occurs in widespread areas of the brain, including the neocortex, hippocampus, striatum and nucleus accumbens. It is likely that the co-localization of mGluR and NMDAR leads to a release of Ca^{2+} from intracellular stores via activation of inositol triphosphat (IP3). LTP can involve the activation of mGluR1 or mGluR5, and can be either of AMPAR-mediated or NMDAR-mediated transmission (Anwyl, 2009). Studies have shown that selective antagonism of either mGluR1 or mGluR5 results in a significant impairment of both induction and maintenance of LTP in freely moving adult rats (Naie and Manahan-Vaughan, 2004; Manahan-Vaughan and Braunewell, 2005; Naie and Manahan-Vaughan, 2005). A study in transgenic mice, including animals with a lack of mGluR5, proposed that mGluR5 plays a regulatory role in NMDAR-dependent LTP (Lu et al., 1997). Another study showed that the animals displayed an abnormal hippocampal LTP expression, and an impaired ability in spatial learning and fear conditioning (Lu et al., 1997; Jia et al., 1998). The theory of a prominent influence of mGluR in LTP received further support by blockage studies which have shown a reduction in LTP duration (Reymann and Matthies, 1989; Izumi et al., 1991).

Another mechanism, also inducing LTP, depends on second messenger cascades. These processes regulate gene transcription and adapt the levels of core proteins at the synapse, such as Ca^{2+}/calmodulin-dependent protein kinase II and protein kinase II. Initiation of this pathway leads to increased concentrations of these two kinases within the dendritic spine, and have been linked to growth in dendritic spine volume and LTP processes, such as the insertion of AMPAR in the synaptic membrane and a phosphorylation of ion channels; this receptor insertion, in turn, enhances the permeability of ions providing the mechanism for long-lasting memory storage.

It remains unclear if LTP expression occurs in a single place only. There is a high probability that several loci exist; they interact with different and specific time courses: Pre-synaptic modification, post-synaptic modifications, extra synaptic changes and morphological modification (Chen et al., 2007; Citri and Malenka, 2008).

It is a matter of time until research will be able to answer this question. Up to now, electrophysiological recordings and biochemical assays have been used to investigate plastic changes. Highly advanced imaging techniques, in combination with fluorescent biosensors, offer us the required spatial and temporal resolution to study LTP in individual synapses. Last year, a report has been published, stating that the examination of cellular processes with single synapse resolution has undoubtedly enhanced our understanding of LTP (Padamsey and Emptage, 2011). In contrast, technical development progressed, but the structure of investigation remained the same, the hippocampus (Padamsey and Emptage, 2011).

1.5 The synaptic homeostasis hypothesis

Five years ago, a promising hypothesis was postulated about sleep wake regulation on a cellular level. It provided a novel conceptual framework to address the still open question about the function of sleep. One of the best established facts in sleep regulation in mammals is that SWA increases in proportion to the duration of prior wakefulness, and progressively decreases during sleep (Borbély, 2001). The synaptic homeostasis hypothesis states that wakefulness is accompanied by synaptic potentiation in a large fraction of cortical circuits, and that sleep is necessary to renormalize the synaptic weight and consequently restore the synaptic balance (Fig. 8) (Tononi and Cirelli, 2003, , 2006). In other words, according to the hypothesis, the change in brain plasticity by the synaptic potentiation, which occurs during wakefulness, is tied to the increase in SWA during the subsequent sleep episode, and are even positively related to each other.

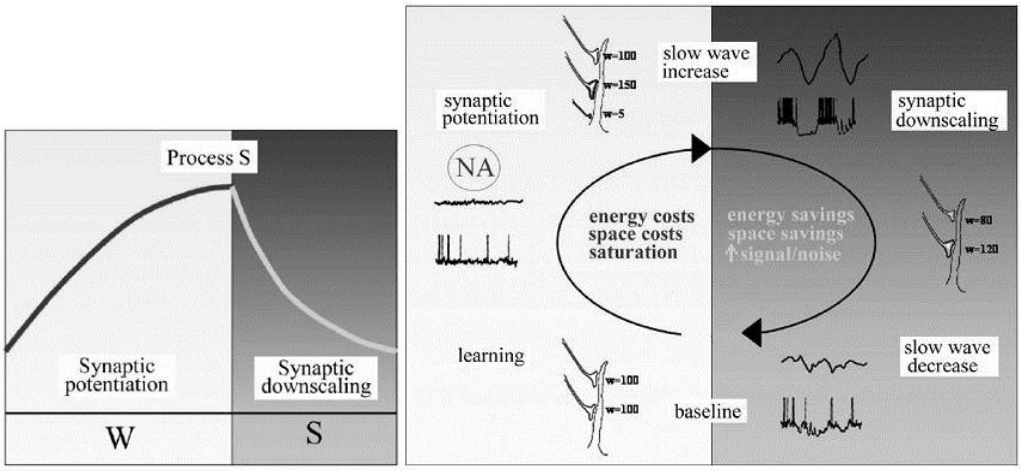

Figure 8: The synaptic homeostasis hypothesis: a schematic diagram.
Left side Synaptic potentiation and downscaling is suggested to be a cellular correlate of process S. **Right side** During wakefulness, when the organism is actively engaging in various waking tasks, strong synaptic activation is accompanied by a change in the neuromodulatory milieu (for example, high levels of noradrenaline (NA), which favors the storage of information by long-term potentiation. This process requires energy and space, and is thought to have a saturation level. The change from the wake- to the sleeping vigilance state causes a change in the neuromodulatory milieu, which triggers the occurrence of high amplitude slow oscillations, reflected in the EEG as SWA. Also the higher amount of synapses is suggested to lead to a higher synchronous cortical firing at the beginning of the night. During the progression of the night downscaling occurs; it is associated with energy saving, but also with space saving and an increased signal-to-noise ratio. At the end of the cycle, the slow oscillation disappears in correspondence to a lower number of synapses. The total amount of synaptic weight is back to baseline level but different to the spines. Thereby the synaptic balance is kept upright. White background: during wakefulness; dark background: during sleep (Tononi and Cirelli, 2006).

By having a closer look at the brain physiology, it was observed that the firing of the noradrenergic system is high during wakefulness, and very low or even absent during sleep (Aston-Jones and Bloom, 1981). These molecules play an important role in the induction of LTP. Experimental lesion of the noradrenergic system led to an impairment in some forms of learning (Robbins and Everitt 1995). Furthermore, plasticity-related genes, such as activity-regulated cytoskeleton-associated protein (Arc), Brain-derived neurotrophic factor (BDNF) or nerve growth factor inducible protein A (NGFI-A), have been suggested to be involved in LTP (Ying et al., 2002; Silva, 2003) and to be expressed preferentially

during wakefulness. In contrast, it could be shown that the expression of LTP-related genes is severely reduced, or even abolished during sleep (Cirelli and Tononi, 2000). To sum up, spontaneous wakefulness is inevitably associated with some LTP-related molecular changes, while sleep is not (Tononi and Cirelli, 2001). A recent study could even show brain plasticity-like features in Drosophila. The testing of three neuronal circuits in Drosophila (ventral lateral neurons, mushroom bodies, lobula plate vertical system) resulted in the finding that synapse size, or number, increases after a few hours of wakefulness, and decreases only if flies were allowed to sleep. The consequences of an enriched environment were both a larger synaptic growth and greater sleep need (Klintsova and Greenough, 1999; Bushey et al., 2011). Flies that were allowed to sleep after the enriched environment experience showed in all morphological parameters a decline to the levels observed in awake flies kept in single tubes (Bushey et al., 2011). Finally, overall levels of synaptic proteins in the fly's brain increase after wake and decrease after sleep (Gilestro et al., 2009). Other techniques have been used to support the prediction of the increase in synaptic weight in the course of normal wakefulness and decline during sleep. Brain metabolism, as measured with water positron emission tomography (PET), showed that blood flow was 18% higher at the end of the waking period than after a night of sleep (Braun et al., 1997). The other approach was taken by recording miniature excitatory postsynaptic currents (mEPSCs) with whole-cell voltage clamp. Frontal cortex slices of mice and rats, which had been awake or asleep, were recorded. Independent of time of day, the outcome revealed an increase in the frequency and amplitude of mEPSCs after waking, and a decrease after sleep. Miniature EPSCs were also decreased after deprivation in the recovery sleep, supporting the suggestion that sleep favors synaptic homeostasis (Liu et al., 2010).

These results suggest that a possible role of sleep in synaptic homeostasis can be shown by different techniques, and even in phylogenetically distant species; it may thus be of general importance. But there are also conflicting results, where

authors showed that the plastity of neurons is enhanced during sleep (Frank et al., 2001; Aton et al., 2009).

1.6. Glutamatergic mechanisms and signalling pathways in wakefulness and sleep, brain plasticity and disease

Glutamate is considered the major excitatory neurotransmitter in the mammalian central nervous system (CNS). It can act through ionotropic (iGluR) and mGluR subtypes. The glutamatergic synapse attracted much attention in research, and knowledge has advanced enormously over the last ten years, primarily through application of cellular electrophysiological and molecular biological techniques to the mGluRs and transporters (GLTs) (Fig. 9). Today, eight subtypes of the mGluRs are identified and classified into three groups, based on their sequence identity, pharmacological properties and preferred signal transduction mechanism. Group I (mGluR1 and mGluR5) is coupled to phospholipase C (PLC), and up-regulates or down-regulates neuronal excitability. These receptors are localized predominantly on postsynaptic membranes (Niswender and Conn, 2010). Furthermore, there is evidence that class I mGluRs, in particular mGluR5, are expressed on astrocytes and post-synaptic membranes of neurons and are critical in signal modulation (van den Pol et al., 1995; Cai et al., 2000; Fellin et al., 2004). Authors claim that glia are well positioned to modulate multiple aspects of synaptic plasticity (Barker and Ullian, 2010). Group II (mGluR2 and mGluR3) and group III (mGluR4, mGluR6, mGluR7 and mGluR8) inhibit adenylat cyclase (AC) and, hence, reduce synaptic transmission. All mGluRs are polypeptides with a putative 7-trans-membrane spanning domain (Pin and Acher, 2002). The activation of mGluRs has been implicated in a variety of CNS functions, such as learning, memory, anxiety, addiction, or pain perception (Swanson et al., 2005; Kauer and Malenka, 2007; Olive, 2009; Xu et al., 2009; Krystal et al., 2010; Jung et al., 2011). One mechanism underlying all these

functions is the synaptic plasticity, which is either beneficially changed or miss-regulated.

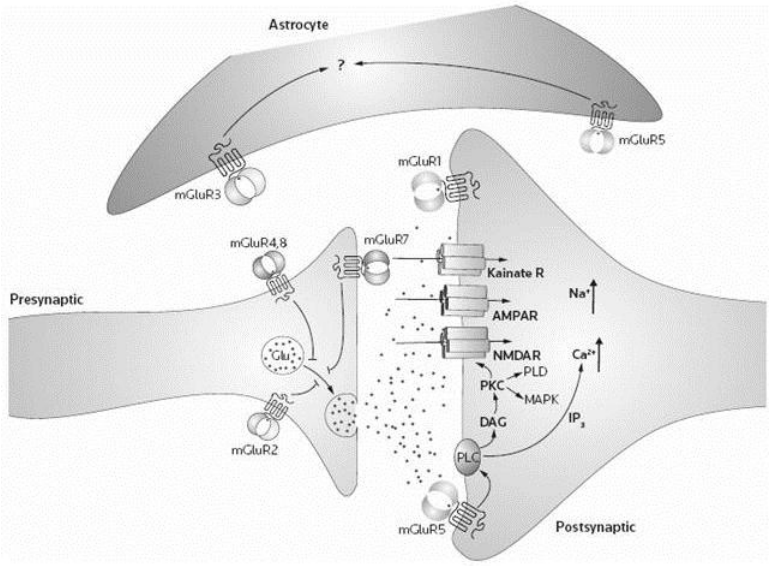

Figure 9: Schematic representation of the glutamatergic transmissions involving iGluRs and mGluRs in a synapse. The filled spheres indicate glutamate, and the question mark indicates that the function of mGluRs on astrocytes has not yet been fully elucidated.
AMPAR: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; DAG: diadylglycerol; iGluR ionotropic glutamate receptor; IP_3: inositol (1,4,5)-triphospate; MAPK: mitogen-activated protein kinase; mGluR: metabotropic glutamate receptor; NMDAR: N-methyl-$_D$-aspartate receptor; PKC: protein kinase C; PLC: phospholipase C; PLD: phospholipase D (Gasparini et al., 2008a).

Multiple proteins interact directly with the intracellular carboxy-terminal tail of each of the mGluR subtypes, and play important roles in their signaling cascade. Some of the best characterized molecules are Homer proteins. Distinct Homer gene and splice variants can differentially regulate localization of mGluR1 and mGluR5 in transfected cells and neurons. The protein contains domains interacting with the last several amino acids of mGluRs, which are critical for receptor activity or that mediate functional responses downstream of the receptors (Tu et al., 1998; Ehrengruber et al., 2004; Shiraishi-Yamaguchi and Furuichi, 2007; Niswender and Conn, 2010). A group of interest in sleep research is the so called Homer family of adaptor proteins, consisting in mammals of three members: Homer1, Homer2 and Homer3; all of which have several isoforms as a result of alternative splicing (Shiraishi-Yamaguchi and Furuichi, 2007). The long

Homer forms (including a coiled-coil domain at the carboxy-terminal) are constitutively expressed, bind to each other through their C-terminal domains, form a tetramer and bind to the target protein (Hayashi et al., 2006). These targets can be mGluR1α/5, IP3R, NMDAR and the actin cytoskeleton through their amino-terminal domains. Homers are highly concentrated at dendritic spines where they are involved in assembling various postsynaptic proteins. Homer1 has, again, several splicing variants whereas Homer1a is of the greatest interest. It has a different protein structure, and a dynamic regulation in response to synaptic activity. Homer1a, which lacks the carboxy-terminal domain, is thought to compete with long Homer forms for binding to target proteins, for example IP3R, thus disrupting the clustering and therefore reducing glutamate-induced intracellular calcium release (Fagni et al., 2000; Shiraishi-Yamaguchi and Furuichi, 2007). In detail: The activation and opening of this IP3R channel protein results from the binding with IP3, assembled by a G-protein coupled receptor regulated PLC-β, which generates IP3 out of phosphatidylinositol 4,5-bisphospat. If the Homer1a concentration decreases in the cell, it leads to increased excitatory effects of mGluR5 activation, whereas an increased concentration and binding to the receptor lead to a reduction in intracellular calcium release. A study by Maret and colleagues found that Homer1a, as well as many other studied proteins, did not show a circadian expression pattern anymore when the animal was sleep deprived; this leads to the assumption that changes in gene expression are in fact sleep-wake dependent. With the hypothesis of Tononi and Cirelli in mind (Tononi and Cirelli, 2003, , 2006), these authors suggested that Homer1a, which is over-expressed after SD, could play a pivotal role in synaptic downscaling by buffering the intracellular calcium release (Maret et al., 2007). Under these circumstances, it is more difficult to excite a cell and initiate, and further elicit an action potential. One needs to keep in mind that not only neurotransmitter and receptors influence sleep, wakefulness and cellular properties, but also the genetic background. It is more and more appreciated that genetic variation is leading to inter-individual differences in vulnerability to sleep loss (Landolt, 2008a; Tafti, 2009).

A further area where the glutamatergic system plays a role is related to synaptic plasticity. Studies performed during the last 10 years revealed a prominent role of mGluRs in the processes of LTP and LTD. Anwyl reported that mGluRs are important in LTP, especially in the neocortex, hippocampus, striatum and nucleus accumbens; yet activation of other receptors may be involved in parallel (Anwyl, 2009). The group around Tononi and Cirelli published several studies on this topic, too. According to the synaptic homeostasis hypothesis, they found on a molecular level that during wakefulness the phosphorylation of AMPARs, CamKII and GSK3β increased in contrast to sleep. During a period of sleep, a significant dephosphorylation of these receptors was observed compared to wakefulness (Vyazovskiy et al., 2008).

A further method used to study the mGluR5 is the immunohistochemical staining. Results show that the distribution of mGluR5 mRNA is widely expressed with the highest density in the olfactory bulb, caudate, putamen, lateral septum, cortex, and hippocampus (Romano et al., 1995). In the cerebellum, only small amounts of mGluR5 mRNA were detected. In addition, a human PET study reported the anterior cingulate cortex (ACC), medial temporal lobe, amygdala, caudate, and putamen as regions with high and moderate mGluR5 density (Ametamey et al., 2007). The same authors suggest that mGluR5 are possibly involved in diseases like anxiety, depression, schizophrenia, Parkinson's, addiction, or pain (Ametamey et al., 2007).

A recent study investigated the effect of major depression on mGluR5 density (Deschwanden et al., 2011). With the method of PET they found lower levels of mGluR5 binding in prefrontal cortex, cingulate cortex, insula, thalamus, and hippocampus. In addition, they observed negative correlations between the mGluR5 availability and depressive symptoms, as assessed with the Beck depression inventory, in all regions. The severity of symptoms was negatively correlated with mGluR5 binding in the hippocampus (bilaterally). Moreover, anxiety symptoms were negatively correlated with mGluR5 binding in thalamus,

orbito-frontal cortex (bilaterally), right frontal polar cortex and left middle cingulate cortex.

A novel field of research emerged by the involvement of mGluR5 as a novel player in the treatment of fragile X syndrome (FXS). It is the most commonly inherited form of mental retardation and a leading known cause of autism (Krueger and Bear, 2010). The syndrome is caused by the repetition of a certain gene sequence on the X chromosome. The repetition results in a failure of the body to express the fragile mental retardation (FMR1) protein, which is required for normal neural development. Studies have examined the phenotypes of FXS in mice. Interbred with mGluR5 knockout animals have revealed that many of the phenotypes of FXS can be rescued when mGluR5 levels are reduced (Dolen et al., 2007). Also genetic reduction of mGluR5 signaling reduced FXS in mice (Bassell and Gross, 2008). Selective mGluR5 negative allosteric modulators are used as treatment of FXS (Bear et al., 2004; Yan et al., 2005; Dolen et al., 2007). This protein is not only of interest for pharmaceutical companies, but also for researchers, since up to now it was believed that the progression of mental retardation diseases cannot be stopped. In 2011, a study conducted in drosophila suggested a link between sleep and FXS (Bushey et al., 2011). The study demonstrated that the fragile-X-mental retardation-1 gene (*Fmr1*) plays an important role in sleep-dependent synaptic renormalization. The authors cite an own study where they showed that Fmr1 protein levels increase in the adult fly brain during wakefulness relative to sleep, independent of time of day or night (Bushey et al., 2009). Thus, waking experience may be sufficient to affect Fmr1 expression even after the end of development.

The role of glutamatergic mechanisms in neurologoical diseases is and was the focus of many efforts to discover ligands as potential therapeutic targets to agonize or antagonize the glutamatergic transmission, and treat these disorders.

1.7 Sleep disorders

Back in 1976, Bedrich Roth was one of the first researcher who influenced and formed the term of idiopathic hypersomnia (IH) (Roth, 1976). Patients with IH were subdivided into a symptomatic and a functional group. This grouping changed over the last 40 years several times and shows the difficulty to categorize this clinically heterogenous group. The main characteristic remained the same over the past years, namely, it is the disabling excessive daytime sleepiness (EDS) (Billiard, 2007). It needs to be mentioned that excessive sleepiness should be separated from fatigue and lack of energy associated with a variety of medical and psychiatric diseases. Today, little is known about the pathophysiology of IH; this is not surprising regarding the little research that was done with this patient group. A second reason is that no animal model of IH is available and, thus, experimental approaches are limited. A third reason is the low prevalence of this condition, which is assumed to be 0.002-0.02% of the general population (Billiard and Dauvilliers, 2001). Approximately 5% of patients in sleep clinics, who complain of daytime sleepiness, are diagnosed with IH (Bove et al., 1994; Bassetti and Gugger, 2000; Sforza et al., 2000). Despite the EDS, further symptoms have been observed and described in recent years. The International Classification of Sleep Disorders (ICSD-2) comprises the present classification of IH (AASM, 2005).

Idiopathic hypersomnia is divided into two diagnostically different subgroups. The first group is referred to as IH with long sleep time. Despite no apparent nocturnal sleep disturbance, this group has three prominent features: Constant excessive daytime sleepiness with unwanted naps which are non-refreshing, prolonged nocturnal sleep, and laborious awakening in the morning and/or after naps (Vernet et al., 2010; Billiard, 2011). The clinical diagnosis comprises six characteristics: A complaint of EDS lasting more than 3 months; more than 10 hours night sleep documented by actigraphy, sleep logs or interview, and sleep drunkenness in the morning; nocturnal polysomnography (PSG) revealing no other causes of daytime sleepiness; short sleep latency and more than 10 hours

night sleep demonstrated by a polysomnogram; sleep latency in the multiple sleep latency test (MSLT) which is less then 8 minutes and fewer then 2 sleep onset REM (SOREM) sleep episodes; hypersomnia is not better explained by any other disorder. The second group is referred to as IH without long sleep time. The clinical diagnosis comprises the same six characteristics noted above with the difference in the second criteria of night sleep time between 6 and 10 hours (AASM, 2005; Billiard, 2007).

The mentioned symptoms may vary in severity and occurrence across the patients. The course of the disease is initially progressive but often stable by the time of diagnosis, which is often several years after the onset. Idiopathic hypersomnia becomes apparent during adolescence or the early twenties, and appears to be a life-long disorder with no tendency to remit spontaneously. To counteract the EDS, stimulants, such as modafinil or antidepressants, are taken by IH, although not all patients respond to the medication. Antidepressants act mainly by blocking the re-uptake of monoamines into the synapses, leading to a higher concentration in the synaptic cleft and to an increased down-stream signaling (Zhou et al., 2007).

Currently, only few polysomnographic studies of IH are available. One study found that the conventional sleep variables, and the cyclic alternation of NREMS and REMS episodes are undisrupted in patients with IH (Fig. 10A) (Bove et al., 1994; Sforza et al., 2000). Further analysis of sleep spindles (SS) revealed that IH had an increased SS density at the beginning and the end of nocturnal sleep, and an increase in number of SS (Bove et al., 1994). Studying δ-activity revealed lower values in the patient group compared to healthy subjects (Fig. 10B) (Sforza et al., 2000); this led to the assumption of a disrupted homeostatic process. A study examining the secretion of melatonin and cortisol hypothesized a disturbed circadian rhythm by demonstration of a decreased melatonin concentration and a 2-h delay phase shift. Cortisol concentrations were similar to healthy controls, though with a 4-h delayed shift (Nevsimalova et al., 2000). To elucidate a

possible dysregulation of homeostatic and/or circadian processes in IH patients, a sleep deprivation study would be beneficial.

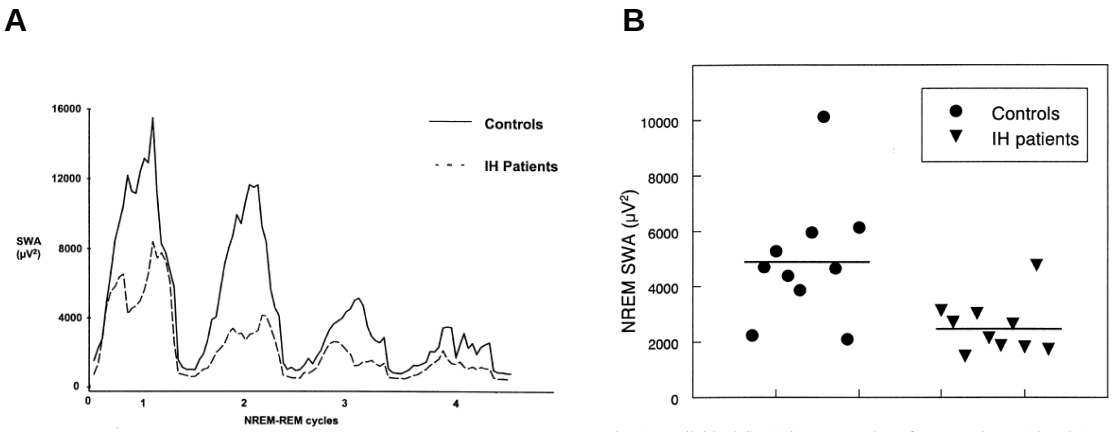

Figure 10: Difference in slow-wave activity (SWA) between IH and control subjects. **A** Dynamics of the SWA over four consecutive sleep cycles in controls (straight lines) and IH patients (dotted line). In the patient groups, SWA was significantly lower during the first two NREM episodes but its temporal decay was preserved. **B** Individual SWA in NREMS for IH patients (triangles) and controls (circles). SWA is significantly lower in the IH patient group (Sforza et al., 2000).

The opposite clinical entity of hypersomnia is insomnia. Most patients with insomnia have trouble falling asleep and experience frequent awakenings during sleep (Roth, 2007). Studies in this group found in the EEG power spectrum increased β-activity (Merica et al., 1998; Perlis et al., 2001), which is assumed to be an index of arousal, and decreased α-activity (Bonnet and Arand, 1994). The same authors, about 20 years later, wrote a review about hyperarousal and insomnia (Bonnet and Arand, 2010). They suggested that insomnia is associated with inappropriate physiological arousal; in particular, a state in which sleep and arousal systems are both simultaneously activated. They wrote that subjects with higher cardiovascular activity, which is an indirect measure of central nervous system arousal, had also higher sympathetic nervous system activation and therefore poorer sleep compared to controls (Bonnet and Arand, 2010).

A related sleep disorder is narcolepsy (NC) which can occur with or without cataplexy. Men and women are equally affected, in contrast to IH, to which

woman are more prone (Lavault et al., 2011). The usual onset of NC lies between 15 and 30 years of age. The typical tetrade of symptoms is EDS, with continuous feeling of sleepiness or sleep attacks; the cataplexy with a sudden bilateral atonia of striated muscles with partial or complete weakness; the hypnagogic or hypnapompic hallucinations; and the sleep paralysis (Bassetti and Aldrich, 1997; Guilleminault and Brooks, 2001; AASM, 2005). Only 10% of patients experience the full tetrade. A frequent polysomnographic feature is the occurrence of sleep onset REMS periods (SOREMs) in the MSLT (Guilleminault et al., 1994; Aldrich et al., 1997).

In many cases it is difficult to clinically differentiate patients with narcolepsy with cataplexy from patients affected by IH without long sleep time (Dauvilliers et al., 2003), although recent studies could show polysomongraphic differences. Authors reported that patients with NC had shorter sleep onset and REMS latencies, lower sleep efficiency and sleep stage 1 percentage, shorter sleep latencies on the MSLT, and a higher amount of SOREMs than patients with IH (Lavault et al., 2011). Furthermore, NC showed significantly shorter sleep latency and REM latency than IH (Takei et al., 2012). A similarity between IH and NC is the response to modafinil, a wake promoting substance. Bastuji and Jouvet reported that modafinil decreased the number of daily naps in 15 out of 18 patients with IH (83%); this is a percentage similar to the one seen in NC (Bastuji and Jouvet, 1988). With the intake of modafinil, the Epworth sleepiness score, a subjective estimation of the probability to fall asleep in a daily situation, was strongly reduced by 4.3 points in IH (Lavault et al., 2011).

A prominent deficit and difference to IH is the loss, or reduced amount of hypocretin (Hrct) cells, also referred to as orexin, in the hypothalamus in NC (Billiard and Dauvilliers, 2001; Mignot et al., 2002). A study could show that the amount of these Hrct neurons and their transmission was reduced (Lin et al., 1999; Mochizuki et al., 2004). Reduced Hrct levels were found in the cerebrospinal fluid (Nishino et al., 2000; Mignot et al., 2002), and, in addition, post mortem brain tissue showed a loss of Hrct cells (Thannickal et al., 2000). Even more interesting are the findings which show that Hrct plays an important

role in stabilizing the state of wakefulness and sleep (Adamantidis et al., 2007; Sakurai, 2007). A recent study even suggests that Hcrt promotes wakefulness whereas melanin concentrating hormone (MCH) induces REMS (Peyron et al., 2011).

Neurotransmitters are essential messengers between distinct brain areas. In the next section, some neurotransmitters and their sites of action will be discussed.

1.8 Anatomical and physiological background of state transition between wakefulness and sleep

Since von Economo's report in 1916, that two distinct brain regions may be responsible for sleep and wakefulness problems, researchers have been interested in finding out what the underlying neuroanatomy and -transmitters are. Von Economo could identify a region between the brainstem and the forebrain that caused prolonged sleepiness, and a second site of lesion in the anterior hypothalamus that caused prolonged insomnia. One review, seven years ago, summarized the complexity of interactions in the brain between neurotransmitters and physiological mechanisms. It discusses the question in which anatomical structure arousals arise, and which neurotransmitters play a role in inducing a solid state of sleep or wakefulness (Saper et al., 2005).

The complexity of the interacting neurotransmitters and their anatomical location are illustrated in more detail in the simplified schema in Figure 11.

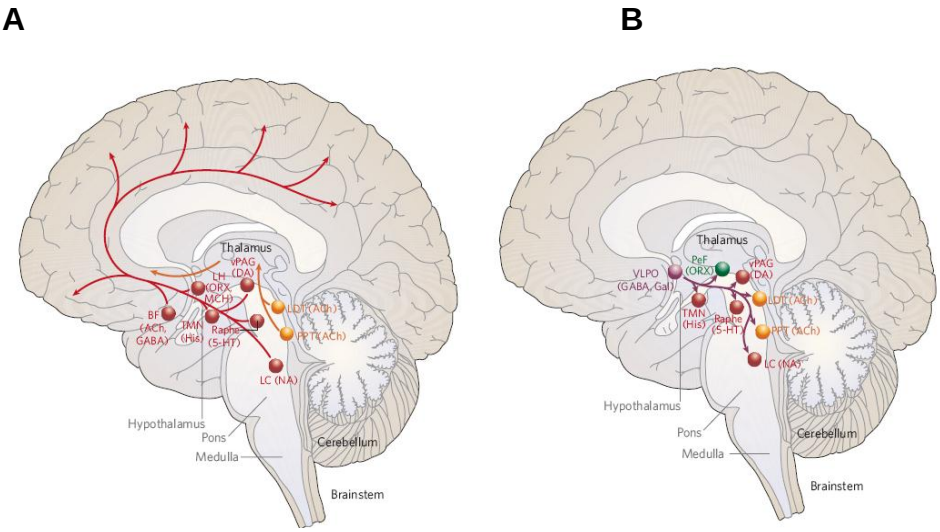

Figure 11: A A Schematic drawing showing some key components of the ascending arousal system. A major input to the relay and reticular nuclei of the thalamus (yellow pathway) originates from cholinergic (ACh) cell groups in the upper pons, the pedunculopontine (PPT), and laterodorsal tegmental nuclei (LDT). These inputs facilitate thalamocortical transmission. A second pathway (red) activates the cerebral cortex to facilitate the processing of inputs from the thalamus. This arises from neurons in the monoaminergic cell groups, including the tuberomammillary nucleus (TMN) containing histamine (His), the A10 cell group containing dopamine (DA), the dorsal and median raphe nuclei containing serotonin (5-HT), and the locus coeruleus (LC) containing noradrenaline (NA). This pathway also receives contributions from peptidergic neurons in the lateral hypothalamus (LHA) containing orexin (ORX) or melanin-concentrating hormone (MCH), and from basal forebrain (BF) neurons that contain γ-aminobutyric acid (GABA) or ACh.

B A schematic drawing showing the key projections of the ventrolateral preoptic neucleus (VLPO) to the main components of the ascending arousal system. It includes the monoaminergic cell groups (red), such as the tuberomammillary nucleus (TMN), the A10 cell group, the raphe cell groups, and the locus coeruleus (LC). It also innervates neurons in the lateral hypothalamus (LHA; green), including the perifornical (PeF) orexin (ORX) neurons, and interneurons in the cholinergic (ACh) cell groups (yellow), the pedunculopontine (PPT), and laterodorsal tegmental nuclei (LDT). 5-HT, serotonin; GABA, γ-aminobutyric acid; gal, galanin; NA, noradrenaline; His, histamine (Saper et al., 2005).

The ascending arousal system (AAS) controls arousal and mental alertness (Steriade, 1996). The AAS was originally referred to as ascending reticular activating system 60 years ago (Moruzzi and Magoun, 1949) which originates in the brainstem and basal forebrain (BF), runs through the midbrain reticular

formation (RF) and innervates the thalamus and cortex (Moruzzi and Magoun, 1949; Jones, 2005b; Saper et al., 2005). RF projection neurons are likely to use glutamate as a neurotransmitter (Jones, 1995), and maintain cortical activation and behavioral arousals of wakefulness (Jones, 2005a). There are two pathways of the AAS: a dorsal and a ventral one (Fig. 11A). The two main regions in the dorsal pathway, which project with mainly cholinergic cells to the thalamus, are the pedunculopontine (PPT) and laterodorsal tegmental nuclei (LDT) (Hallanger et al., 1987). A study in animals showed evidence that the PPT has wake promoting function. The researchers injected glutamate into the PPT of rats and found an increased duration of wakefulness and REMS duration in a dose-dependant manner (Datta et al., 2001). The activation of the thalamus by the PPT and LDT facilitates further thalamocortical transmission. The ventral pathway arises from several regions in the brainstem and caudal hypothalamus, bypasses the thalamus and projects to the BF and the cerebral cortex. These regions contain monoaminergic cell groups and include the locus coeruleus (LC, noradrenalin), the raphe nucleus (RN, serotonin), and the tuberomamillary nucleus (TMN, histamine). The ventral pathway is further activated by Hcrt cells originating in the lateral hypothalamus (Saper et al., 2005). Many of the Hcrt neurons contain glutamate (Torrealba et al., 2003). They send axons to the entire cerebral cortex, to the brainstem and BF, with particular input to TMN and the LC (Peyron et al., 1998). Hcrt neurons are suggested to play an important role in stabilizing wakefulness and sleep (Adamantidis et al., 2007; Sakurai, 2007).

The ‘flip-flop switch’ model of sleep and wakefulness is presented in Figure 12. This model claims that wake and sleep are two distinct and consecutive vigilance states. Wakefulness is primarily initiated and stabilized by monoaminergic and cholinergic neurotransmission in the upper brain stem. The ventrolateral preoptic nucleus (VLPO) plays a key role in this model. Its action is either suppressed by the TMN, LC, and RN – with the effect of wakefulness – or, in contrast, the VLPO is highly active and suppresses these regions, resulting in the state of sleep.

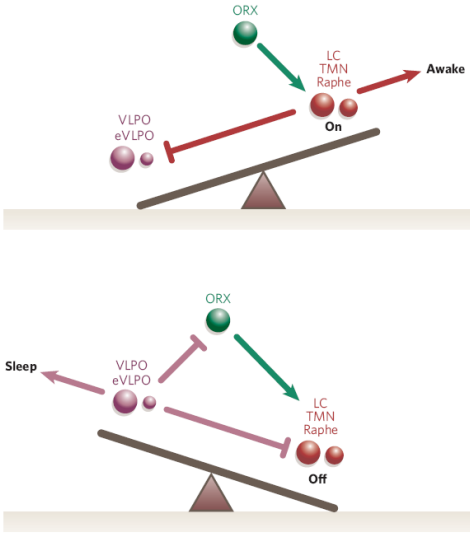

Figure 12: A schematic diagram of the flip-flop switch model. During wakefulness (**upper panel**), the monoaminergic nuclei (red) inhibit the ventrolateral preoptic nucleus (VLPO; purple), thereby relieving the inhibition of the monoaminergic cells, and that of the orexin (ORX) neurons (green), and the cholinergic pedunculopontine (PPT) and laterodorsal tegmental nuclei (LDT; yellow). Because the VLPO neurons do not have orexin receptors, the orexin neurons serve primarily to reinforce the monoaminergic tone, rather than directly inhibit the VLPO on their own. During sleep (**lower panel**), the firing of the VLPO neurons inhibits the monoaminergic cell groups, thereby relieving their own inhibition. This also allows it to inhibit the orexin neurons, further preventing monoaminergic activation that might interrupt sleep. The direct mutual inhibition between the VLPO and the monoaminergic cell groups forms a classic flip-flop switch, which produces sharp transitions in state, but is relatively unstable. The addition of the orexin neurons stabilizes the switch. 5-HT, serotonin; ACh, cholinergic; eVLPO, extended ventrolateral preoptic nucleus; GABA, γ-aminobutyric acid; gal, galanin; LC, locus coeruleus; NA, noradrenaline; PeF, perifornical; REM, rapid eye movement; TMN, tuberomammillary nucleus (Saper et al., 2005).

There are two components which play an important role in the state of wakefulness and sleep: AAS and VLPO. Recordings from VLPO neurons across the sleep-wake cycle, including sleep deprivation, showed that their firing rates increased with sleep EEG slow-waves (Szymusiak et al., 1998). These findings support the hypothesis of Figure 12 of a VLPO firing during sleep.

These observations suggest that the reciprocal activities and interactions of different populations of wake- and sleep-promoting neurons are distinct, and that

each may contribute differently and at distinct points in time to the states of wakefulness or sleep. Hence, not one of the mentioned systems itself appears to be absolutely necessary for wakefulness or sleep, although each contributes in a unique way to its generation and maintenance (Jones, 2005b).

The main message concerning IH and NC is that a well-defined state transition is missing; this is reflected by strong sleep inertia in the morning in IH, and sleep attacks during the day in NC. Possible mechanisms and models are discussed in the last chapter of this work.

1.9 The aim of the thesis

The general purpose of the current thesis is to investigate mechanisms regulating sleep and wakefulness. Physiological, biochemical, and behavioral measures were studied.

To challenge the homeostatic process of sleep regulation, we performed sleep deprivation (SD) studies (see study design in appendix II & III). With this intervention, we first investigated a possible participation of mGluR5 receptor density in the homeostatic process in healthy individuals. Secondly, we used the same paradigm to investigate possible changes in the build-up of sleep need and alertness in patients with IH.

In the first project (Chapter 2), mGluR5 density in the human brain was studied with the method of PET, aiming at enlarging the knowledge about receptor availability in response to prolonged wakefulness. Studies suggest that the brain undergoes plastic changes during wakefulness and sleep, namely, LTP and LTD respectively. Our hypothesis was that SD would lead to an increased mGluR5 density in healthy male volunteers, since studies have suggested that sleep need is linked to neural plasticity. Studies have also shown that wakefulness is associated with synaptic potentiation that is another mGluR-mediated process.

We compared PET data of a control condition, where the subjects could sleep the night before the scan, with data collected after a night without sleep.

The purpose of the second project (Chapter 3) was to study physiological sleep-wake regulation in patients with IH. We aimed to obtain a deeper insight into the pathophysiology of this disease, which is characterized by excessive daytime sleepiness of unknown origin. Studies have shown that increasing sleep pressure during prolonged wakefulness is reflected by θ-activity in the waking EEG (wEEG), whereas during the subsequent recovery sleep the enhanced homeostatic process is reflected by predictable changes in δ-activity in the sleep EEG. We were interested to see if we could observe frequency-specific differences in EEG spectral power in the course of prolonged wakefulness. Furthermore, we studied and compared sleep parameters between IH patients and healthy controls in the recovery night after sleep deprivation. We addressed three specific questions: Do patients with IH have reduced tolerance to sleep pressure? Do they have an altered build-up of sleep need? Or, is there a disturbed interaction of the homeostatic and circadian processes?

All data of this thesis were recorded in the sleep laboratory of the Institute of Pharmacology and Toxicology at the University of Zürich. I contributed to the data collection and the analyses of cognitive tasks, questionnaires, PET data, and EEG data. In preparation of the publications reporting the results described in Chapters 2 and 3, I drafted the first versions of parts of the manuscripts and revised them with the help of the co-authors.

Chapter 2

Increased availability of metabotropic glutamate receptor subtype 5 in human brain after one night without sleep

Katharina Hefti [1*], Sebastian C. Holst [1,8*], Judit Sovago [2], Valérie Bachmann [1,8], Alfred Buck [3,7,8], Simon M. Ametamey [4,7], Milan Scheidegger [5,6], Thomas Berthold [3], Baltazar Gomez-Mancilla [2], Erich Seifritz [5,7,8], and Hans-Peter Landolt [1,7,8]

[1] Institute of Pharmacology & Toxicology, University of Zürich, Zürich, Switzerland

[2] Novartis Institutes for Biomedical Research, Basel, Switzerland

[3] Division of Nuclear Medicine, University Hospital Zürich, Zürich, Switzerland

[4] Center for Radiopharmaceutical Sciences of ETH, PSI & USZ, Zürich, Switzerland

[5] Clinic of Affective Disorders and General Psychiatry, Psychiatric University Hospital, Zürich, Switzerland

[6] Institute for Biomedical Engineering, University & ETH Zürich, Zürich, Switzerland

[7] Neuroscience Center Zürich, University of Zürich & ETH Zürich, Zürich, Switzerland

[8] Zürich Center for Integrative Human Physiology (ZIHP), University of Zürich, Zürich, Switzerland

* *These authors contributed equally to this work.*

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Abstract

Background: Sleep deprivation ("wake therapy") provides rapid clinical relief in many patients with major depressive disorder (MDD). Changes in glutamatergic neurotransmission may contribute to the antidepressant response, yet the exact underlying mechanisms are unknown. Metabotropic glutamate receptors of subtype 5 (mGluR5) are importantly involved in modulating glutamatergic neurotransmission and neuronal plasticity. The density of these receptors is reduced in the brain of patients with MDD, particularly in brain structures involved in regulating wakefulness and sleep. We hypothesized that prolonged wakefulness would increase mGluR5 availability in human brain.

Methods: mGluR5 binding was quantified with positron emission tomography (PET) in 22 young healthy men who completed 2 experimental blocks separated by one week. Two PET examinations were conducted in randomized, cross-over fashion with the highly selective radioligand, ^{11}C-ABP688, once after 9 hours (sleep control) and once after 33 hours (sleep deprivation) of controlled wakefulness. ^{11}C-ABP688 uptake was quantified in 13 volumes of interest with high mGluR5 expression and presumed involvement in sleep-wake regulation.

Results: Sleep deprivation induced a global increase in mGluR5 binding when compared to sleep control ($p < 0.006$). In anterior cingulate cortex, insula, medial temporal lobe, parahippocampal gyrus, striatum, and amygdala, this increase correlated significantly with the sleep deprivation-induced increase in subjective sleepiness.

Conclusions: This molecular imaging study demonstrates that cerebral mGluR5 availability is increased after a single night without sleep. Given that modulators of mGluR5 have antidepressant properties, further research is warranted to examine whether this mechanism is involved in the potent antidepressant effect of ‘wake therapy’.

Introduction

The regulation of sleep and mood are tightly associated. Disrupted sleep is an important diagnostic criterion and risk factor for major depressive disorder (MDD) (Baglioni et al., 2010). Insomnia symptoms often precede the onset of depression by several months, are resistant to treatment, and increase the risk of relapse in remitted patients. Intriguingly, whereas sleep-wake disturbances are highly prevalent in MDD, sleep deprivation ("wake therapy") provides rapid clinical relief in many patients (Bunney and Bunney, 2011). Changes in glutamatergic neurotransmission have been proposed to contribute to the antidepressant response, but the exact underlying mechanisms are unknown.

Metabotropic glutamate receptors including metabotropic glutamate receptors of subtype 5 (mGluR5) play an important role in regulating glutamatergic neurotransmission (Sanacora et al., 2008). The density of mGluR5 is reduced in various cortical and subcortical brain regions in patients with MDD when compared to healthy controls (Deschwanden et al., 2011). These receptors are present on post-synaptic neurons and glia cells and contribute to both long-term depression (LTD) as well as long-term potentiation (LTP) (Ayala et al., 2009; Izumi and Zorumski, 2012). Moreover, they are involved in sleep-wake related postsynaptic plasticity in rats (Tadavarty et al., 2011), and interact directly or indirectly with different molecular markers of sleep-wake regulation in animals and humans. These markers include Homer 1a (Franken et al., 2001; Maret et al., 2007), fragile X mental retardation protein (FMRP) (Bushey et al., 2009, , 2011; Hays et al., 2011), brain-derived neurotrophic factor (BDNF) (Faraguna et

al., 2008; Bachmann et al., 2012) adenosine deaminase (Okada et al., 2003; Bodenmann et al., 2011) and the adenosine A_{2A} receptor (ADORA2A) (Gallopin et al., 2005; Bodenmann et al., 2011).

Prolonged wakefulness not only affects mood and other daytime functions including sleepiness, but also elevates sleep need in a recovery night when compared to a baseline night. The most reliable markers of sleep need are the amount of slow-wave sleep (SWS) and EEG slow-wave activity (SWA) in non-rapid-eye-movement sleep (NREMS) (Achermann and Borbély, 2011). Functional brain imaging studies consistently suggest that ventro-medial prefrontal cortex, basal forebrain, insula, anterior cingulate cortex, striatum, parahippocampal gyrus, precuneus, and other regions, are importantly involved in the regulation of slow-waves and NREMS (Maquet et al., 1997; Dang-Vu et al., 2008; Murphy et al., 2009). Interestingly, mGluR5 are preferentially expressed in most of these brain regions (Gasparini et al., 2008a, 2008b). In addition, they are important for shaping the EEG slow oscillation in NREMS (Blethyn et al., 2006) and synchronized “θ-mode” network activity in wakefulness (Cobb et al., 2000). Based on this convergent evidence for a possible involvement of mGluR5 in sleep-wake regulation, we aimed to quantify mGluR5 availability in rested and sleep-deprived state in humans.

We used the recently developed, selective mGluR5 radioligand, ^{11}C-ABP688, to directly visualize mGluR5 availability in the living human brain (Ametamey et al., 2006; Ametamey et al., 2007). We quantified in healthy adults ^{11}C-ABP688 uptake in sleep control and sleep deprivation conditions in 13 brain

regions with high mGluR5 expression and presumed involvement in sleep-wake regulation. Given the recent observation that mGluR5 density is reduced in depressed patients (Deschwanden et al., 2011) and the suggested role of mGluR5 in sleep-wake related postsynaptic plasticity (Tadavarty et al., 2011), we predicted that prolonged wakefulness may increase mGluR5 availability in the brain.

Materials and methods

Study participants

The study protocol and all experimental procedures were approved by the cantonal and federal authorities for research on human subjects, and carried out in accordance with the Declaration of Helsinki (1964).

Twenty-two healthy young men completed the 2-week study after giving written informed consent. All volunteers were extensively screened for their medical history and psychological state. A pre-study screening night was performed in the sleep laboratory to exclude subjects with unknown sleep disturbances and low sleep efficiency (< 85 %). All participants were non-smokers, right-handed, abstinent from any medication or illicit drugs, and did not cross time zones or do shift work during the last 3 months prior to the experiment. Validated questionnaires to assess life-style and demographic characteristics demonstrated the presence of a healthy study sample (Table 1).

Table 1. Demographic characteristics of study participants.

	Mean ± SD
Age (years)	23.4 ± 2.1
Body-mass index (kg/m^2)	22.1 ± 1.9
Alcohol consumption (drinks/week)	3.5 ± 3.3
Caffeine consumption (mg/day)	153.5 ± 160.9
Diurnal preference	49.7 ± 8.4
Daytime sleepiness	7.1 ± 3.3
Trait anxiety	34.5 ± 7.0
Depression score	3.2 ± 3.8
Eysenck Personality Traits	
Extraversion	8.6 ± 2.8
Neuroticism	3.7 ± 2.8
Lie scale	2.5 ± 1.9
Psychoticism	3.1 ± 1.4
Cloninger Personality Traits	
Novelty seeking	14.7 ± 5.6
Harm avoidance	11.8 ± 4.7
Reward dependence	16.0 ± 5.2

Twenty-two male participants completed the study; one participant had to be excluded from PET analyses because of strong head movement. German versions and validated German translations of questionnaires were used to assess life style and personality traits. Caffeine consumption was estimated based on the following average caffeine contents per serving: Coffee: 100 mg; Ceylon or green tea: 30 mg; Cola drink: 40 mg (2 dl); Energy drink: 80 mg (2 dl); Chocolate: 50 mg (100 g). Diurnal preference: Horne-Östberg Morningness-Eveningness Questionnaire (Horne and Östberg, 1976); daytime sleepiness: Epworth Sleepiness Scale (Bloch et al., 1999); trait anxiety: State-Trait Anxiety Inventory (Spielberger et al., 1970); depression score: Beck Depression Inventory (Beck et al., 1961); personality traits: Eysenck Personality Questionnaire (Francis et al., 2006) and Cloninger's Tridimensional Personality Questionnaire (Weyers et al., 1995).

Pre-study procedures and experimental protocol

Three days before each study block, participants abstained from caffeine and alcohol, and adhered to a 16-hour wake/8-hour sleep schedule. Caffeine-, alcohol- and sleep logs, breath-alcohol measurements upon arrival in the laboratory, and wrist-actigraphy were used to verify adherence to these instructions.

All subjects completed a sleep control and a sleep deprivation condition in randomized, cross-over fashion. Both experimental conditions consisted of

polysomnographically-recorded adaptation, baseline, and recovery nights (time in bed: 23:30-07:30). In the sleep deprivation condition, the baseline night was followed by 40 hours continuous wakefulness during which the participants were under constant supervision of the research team. All subjects underwent 2 PET examinations with ^{11}C-ABP688 to quantify mGluR5 availability in the brain (Division of Nuclear Medicine, University Hospital Zürich). These assessments occurred in random order, either ~ 9 or ~ 33 hours after awakening from the baseline night. The time-of-day of the PET examinations in sleep control (16:36 ± 9 min) and sleep deprivation (16:34 ± 8 min) conditions did not differ ($p > 0.4$).

Magnetic resonance (MR) and positron emission tomography (PET) image acquisition

A T1-weighted, whole-brain, 3D-MR image (resolution: 1 x 1 x 1 mm) was obtained for each subject (Philips Achieva 3T whole-body MR unit equipped with T/R head coil), to exclude morphological abnormalities and as anatomical standard for the quantification of the PET images.

Tracer synthesis and PET brain imaging with ^{11}C-ABP688 using a bolus/infusion protocol were performed as previously described (Ametamey et al., 2006; Ametamey et al., 2007; Burger et al., 2010; Deschwanden et al., 2011). Catheters were placed into the antecubital veins of each participant's arms, one for tracer injection and one for blood sampling. Venous blood was collected at 42 and 58 min after the start of the bolus infusion.

All PET examinations were performed in 3D-mode on a DVCT PET/CT scanner (GE Healthcare) or a DSTx PET/CT scanner (GE Healthcare). A low-dose computer tomography scan was performed before the PET examination for photon attenuation correction. The emission scans were initiated simultaneously with the start of the injection of 563.66 ± 20.35 MBq of radioligand using an infusion pump (PERFUSOR FM, BRAUN Medical); 47.6 % of the total activity (291 ± 7.1 MBq) was injected as a bolus over 2 min (K_{bol} = 53 min) and the rest continuously infused over 58 min (280.82 ± 6.84 MBq). The specific activity of ^{11}C-ABP688 at end of synthesis equaled 128.5 ± 10.4 GBq/µmol (range: 61 - 289 GBq/µmol). Twenty frames were collected during the 60-min protocol (10 x 60 s and 10 x 300 s). The images were reconstructed using filtered back projection and displayed over 47 trans-axial slices. Using a 128 x 128 matrix the resulting voxel size was 2.3 x 2.3 x 3.2 mm.

Subjects were instructed to not fall asleep during image acquisition and the EEG was simultaneously recorded according to established procedures (Bodenmann et al., 2009b; Bodenmann et al., 2011), to verify wakefulness during the PET examinations. As soon as sleep-like EEG activity was noted, they were alerted via an intercom. Direct contact with the subjects was avoided, to minimize movement artifacts.

Image processing and quantification

Image processing consisted of within-subject motion correction by realigning the average of frames 17-19 to the average of frames 2-10 (rigid

matching), spatial normalization of averaged frames 17-19 to the Montreal Neurological Institute (MNI) template brain for definition of volumes of interest (VOI). Besides, the averaged frames 17-19 of the PET images were also co-registered to the corresponding MR image to delineate the cerebellum (Burger et al., 2010). These steps were performed with the PMOD® software package, version 3.1 (PMOD Technologies, Zürich, Switzerland). Standard VOIs for the MNI template brain available in PMOD were used to measure radioactivity concentration in the normalized PET images (Tzourio-Mazoyer et al., 2002). Because PMOD provides no single VOI for cerebellum, this region was defined manually in each subject on the MR image and subsequently transferred to the corresponding PET images, to measure radioactivity concentration in this region. Tissue time activity curves (TAC) were generated for cerebellum, superior frontal cortex, putamen and thalamus in both hemispheres, to confirm that steady state of receptor binding was reached in frames 17-19 (45-55 min) of image acquisition.

The average radioactivity concentration between 45-55 min was calculated in each VOI ($C_{t[VOI]}$). Because some venous blood metabolite analyses were unreliable due to technical difficulties, regional V_t values could not be obtained. Instead, quantification of the PET images was done by dividing the regional radioactivity concentration values with the corresponding value in the cerebellum ($C_{t[Cb]}$), to obtain V_{norm} ($V_{norm} = C_{t[VOI]}/C_{t[Cb]}$). This method was successfully used in previous studies to quantify mGluR5 availability in the brain (Burger et al., 2010; Deschwanden et al., 2011). Furthermore, a preclinical study

showed that ^{11}C-ABP688 binding in the cerebellum is negligible and that this region can thus be used as a reference region (Elmenhorst et al., 2010).

Behavioral and cognitive effects of sleep deprivation

To document that sleep deprivation was successful, validated questionnaires were employed before each PET scan to assess subjective sleepiness (Karolinska Sleepiness Scale) (Akerstedt and Gillberg, 1990), state anxiety (State Trait Anxiety Inventory) (Spielberger et al., 1970), and affective state (Profile of Mood States) (McNair et al., 1981). Approximately 3 hours before each scan, the subjects also completed a cognitive test session consisting of the psychomotor vigilance task (PVT) (Dinges and Powell, 1985) and the Deese-Roediger-McDermott (DRM) false memory paradigm (Deese, 1959; Roediger and McDermott, 1995). To approach a normal distribution, mean reaction time (RT) on the PVT was expressed as speed (1/RT) and the number of lapses (RT > 500 ms) was transformed by ($\sqrt{x}+\sqrt{[x+1]}$).

Cortisol concentration in saliva

Immediately prior to each PET examination, all study participants provided a saliva sample to determine the cortisol concentration as a measure of acute stress. Unstimulated saliva was collected by placing a salivette® (Sarstedt, Sevelen, Switzerland) under the tongue and keeping the head slightly inclined for 1-2 min. The samples were stored at -20 °C until the biochemical analysis took

place. The saliva was centrifuged at 3000 rpm for 5 min before free cortisol was analyzed using an immunoassay with time-resolved fluorescence detection (Dressendorfer et al., 1992).

Statistical analyses

All statistical analyses were performed with SAS® 9.1 software (SAS Institute, Cary, NC). Mixed-effect analysis of variance (ANOVA) models included the factors 'condition' (sleep control, sleep deprivation), 'region' (13 VOIs), and 'hemisphere' (left, right), as well as their interactions. Two-tailed, paired *t*-tests were conducted to localize significant differences. To limit the number of comparisons and to control for type-I errors, analyses of PET data were restricted to the pre-defined VOIs and the significance level was set at $\alpha < 0.0038$ (Bonferroni correction: $\alpha = 0.05/13$). To estimate the possible associations between the effects of sleep deprivation on mGluR5 availability and changes in behavioral and cognitive variables, regression analyses were performed and Spearman rank correlation coefficients were calculated. If not stated otherwise, only significant results are reported.

Results

Regional uptake of ^{11}C-ABP688 in human brain

Axial, sagittal and coronal views of ^{11}C-ABP688 binding in the brain of one single individual in sleep control and sleep deprivation conditions are presented in Fig. 1. Consistent with previous studies (Ametamey et al., 2007; DeLorenzo et al., 2012), regional uptake of ^{11}C-ABP688 reflected the known distribution of mGluR5 with most pronounced binding in anterior cingulate, insula, medial temporal lobe, medial prefrontal cortex, striatum and amygdala. Lower radioligand binding was present in thalamus and substantially lower in cerebellum, which was used to calculate the normalized volumes of distribution (V_{norm}). Visual inspection of TAC in distinct brain regions confirmed that a steady state of ^{11}C-ABP688 uptake was reached 45 min after tracer injection in all participants and scans.

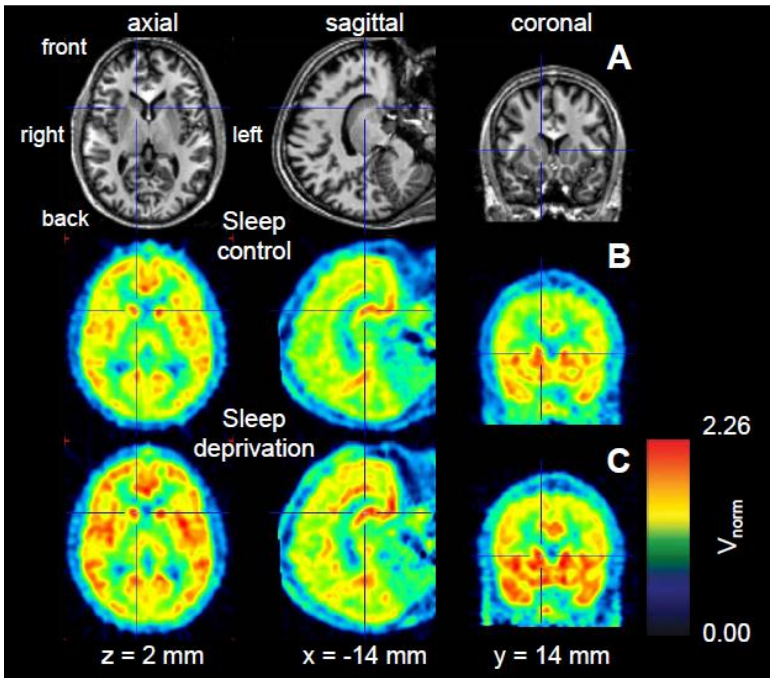

Figure 1. Axial, sagittal and coronal views of ^{11}C-ABP688 binding in a representative individual. (**A**) Magnetic resonance image (MRI) template for anatomical reference. (**B**) Color-coded normalized volumes of distribution (V_{norm}) of ^{11}C-ABP688 after ~ 9 hours of wakefulness (sleep control condition). (**C**) Color-coded V_{norm} of ^{11}C-ABP688 after ~ 33 hours of wakefulness (sleep deprivation condition). The crosshair was placed in the right caudate nucleus (coordinates according to the Montreal Neurological Institute brain atlas: -14, 14, 2).

Sleep deprivation increases mGluR5 availability in human brain

Regional V_{norm} values of ^{11}C-ABP688 in sleep control and sleep deprivation conditions are shown in Fig. 2. Prolonged wakefulness increased global V_{norm} by 3.5 ± 1.1 % when compared to control (1.50 ± 0.02 vs. 1.55 ± 0.02, $p < 0.006$). Mixed-model ANOVA with the factors 'condition', 'region' (volumes of interest [VOIs]) and 'hemisphere' revealed highly significant main effects of 'condition' ($F_{1,20} = 184.5$, $p < 0.0001$) and 'region' ($F_{25,500} = 257.6$, $p < 0.0001$), yet no significant effect of 'hemisphere' ($F_{1,20} = 0.1$, $p > 0.7$). Thus, the data of left and right hemispheres were averaged. The increase in mGluR5 binding was significant in anterior cingulate cortex (3.9 ± 1.3 %, $p < 0.0006$), insula (4.5 ± 1.3 %, $p < 0.0001$), medial temporal lobe (4.2 ± 1.0 %, $p < 0.0005$), parahippocampal gyrus (4.6 ± 1.1 %, $p < 0.0002$), striatum (4.8 ± 1.4 %, $p < 0.0003$), and amygdala (4.3 ± 1.2 %, $p < 0.002$). No VOI showed a reduction in ^{11}C-ABP688 binding after sleep deprivation when compared to control.

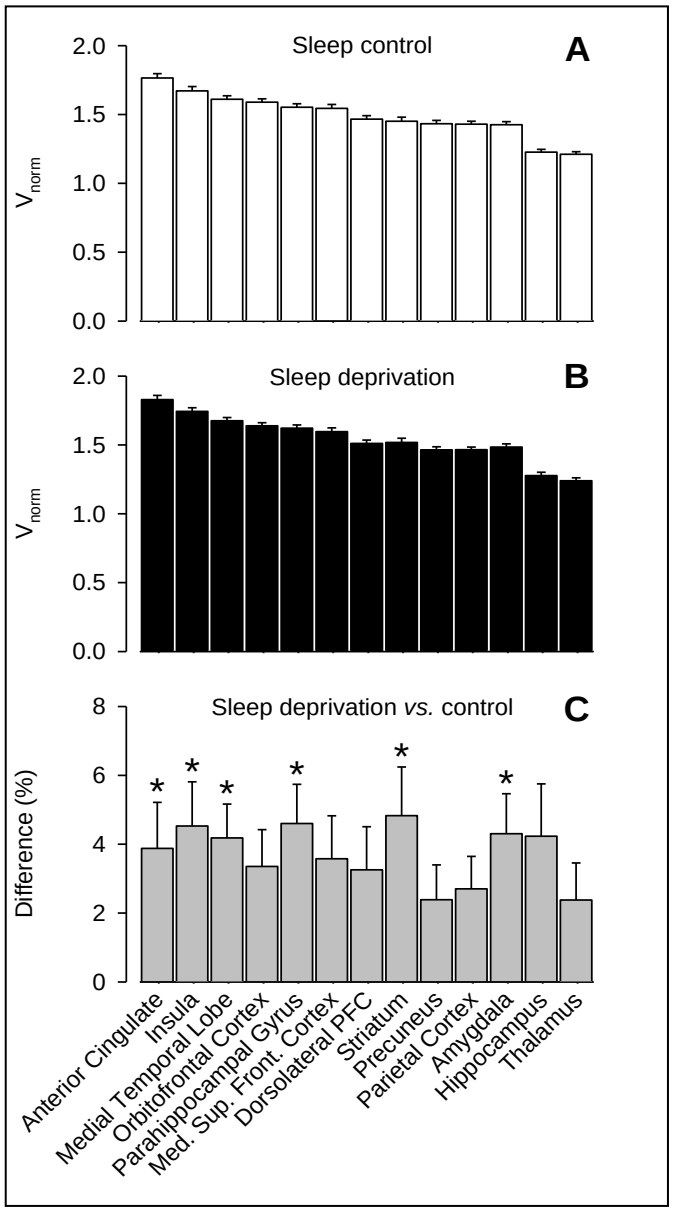

Figure 2. Regional differences in mGluR5 density and effect of sleep deprivation. (**A**) Normalized volumes of distribution (V_{norm}) of ^{11}C-ABP688 uptake in 13 regions of interest after ~ 9 hours of wakefulness (sleep control). (**B**) V_{norm} after ~ 33 hours of wakefulness (sleep deprivation). (**C**) Percent difference in V_{norm} between sleep deprivation and sleep control conditions. The two measurements occurred in random order and were separated by one week. Data represent means + 1 SEM (n = 21). Asterisks indicate significant differences between sleep deprivation and sleep control conditions ('condition': $p < 0.0038$). Med. Sup. Front. Cortex = medial superior frontal cortex; PFC = prefrontal cortex.

Increase in ^{11}C-ABP688 binding correlates with increase in subjective sleepiness

Sleep deprivation impaired subjective state and cognitive performance. Sleepiness, state anxiety, fatigue, lapses on the psychomotor vigilance task (PVT), and false alarms on the Deese-Roediger-McDermott false memory paradigm were increased after prolonged wakefulness when compared to control (Table 2). By contrast, vigor was reduced and PVT reaction times were prolonged.

Table 2. Behavioral and cognitive effects of sleep deprivation.

	Sleep control	Sleep deprivation	p <
Karolinska Sleepiness Scale	3.13 ± 0.28	5.04 ± 0.43	**0.001**
State Anxiety Inventory	36.45 ± 2.07	40.73 ± 2.47	**0.001**
Profile of Mood States (POMS)			
Fatigue	5.77 ± 1.49	13.86 ± 2.22	**0.001**
Vigor	18.54 ± 1.35	10.54 ± 1.38	**0.001**
Depression/Anxiety	3.32 ± 1.42	4.14 ± 2.08	0.506
Irritability	0.91 ± 0.53	1.68 ± 0.72	0.284
Psychomotor vigilance task			
Mean reaction speed (s^{-1})	3.79 ± 0.07	3.50 ± 0.07	**0.001**
Lapses (transformed)	2.29 ± 0.25	4.71 ± 0.62	**0.001**
Deese-Roediger-McDermott Paradigm			
False alarms	5.25 ± 0.99	8.40 ± 0.86	**0.001**
False memory	12.25 ± 0.55	13.80 ± 0.68	0.067
Hit rate	40.35 ± 1.51	41.15 ± 1.80	0.638

Values represent means ± SEM (n = 22). P-values refer to 2-tailed, paired *t*-tests. Significant differences between sleep control and sleep deprivation conditions are highlighted in bold.

Correlation analyses between the sleep deprivation-induced change in subjective sleepiness and the percent change in mGluR5 availability revealed consistent associations in all brain regions showing a significant increase in ^{11}C-ABP688 binding. In other words, those subjects who were most affected by sleep deprivation exhibited the largest increase in mGluR5 binding in anterior cingulate cortex, insula, medial temporal lobe, parahippocampal gyrus, striatum and amygdala (Fig. 3). A similar association was also found for fatigue on the Profile of Mood States questionnaire and mGluR5 availability in medial temporal lobe ($r_p = 0.43$, $p = 0.053$; $r_s = 0.45$, $p < 0.04$; $n = 21$). The other behavioral and cognitive effects of sleep deprivation revealed no consistent correlation with the changes in mGluR5 binding in the significant VOIs.

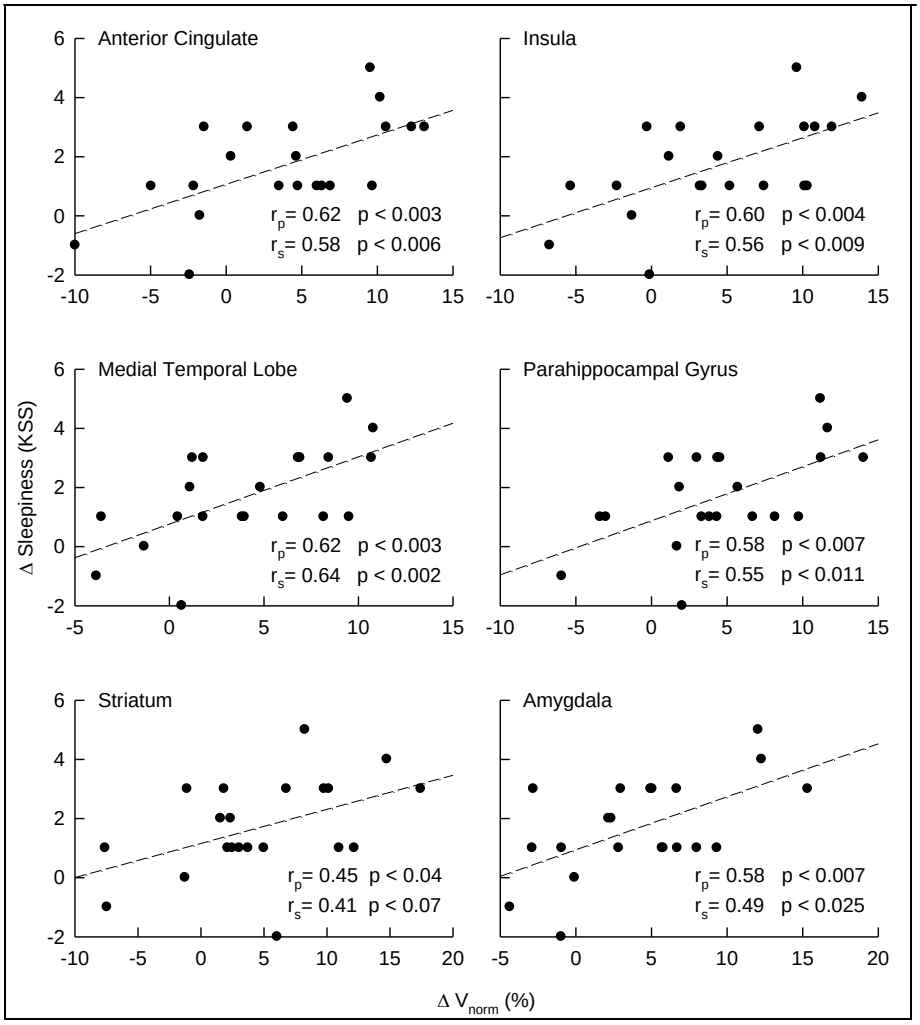

Figure 3. Relationship between the individual subjects' difference in subjective sleepiness and the difference in mGluR5 availability between sleep deprivation and sleep control conditions. Individual percent changes in normalized volumes of distribution (V_{norm}) of ^{11}C-ABP688 in those brain regions that showed a significant increase after sleep deprivation are illustrated. A linear regression line was fitted through the 21 individual data points. KSS = Karolinska Sleepiness Scale; r_p = Pearson's product-moment correlation coefficient; r_s = Spearman rank correlation coefficient.

Cortisol concentration in saliva

Prolonged wakefulness tended to slightly increase salivary cortisol concentration when compared to the sleep control condition (9.4 ± 0.6 *vs.* 7.0 ±

0.6 mmol/l, $p < 0.08$). The individual changes in salivary cortisol were not associated with individual changes in mGluR5 binding.

Discussion

This *in vivo* molecular imaging study in humans reveals the first evidence for increased cerebral mGluR5 availability after prolonged wakefulness. After a single night without sleep, binding of ^{11}C-ABP688 was significantly increased on a global level, and in distinct brain regions that were previously shown to reflect physiological changes after sleep deprivation (for recent review, see (Dang-Vu et al., 2010)). These effects of short-term sleep loss are intriguing. The expression of G-protein coupled receptors (GPCR) on the cell surface is tightly regulated and provides a powerful mechanism of signal amplification (Nelson and Cox, 2008). A recent study demonstrated that regional ^{11}C-ABP688 binding may reflect mGluR5 protein expression (Deschwanden et al., 2011). Because mGluR5 density is reduced in major depression (Deschwanden et al., 2011), our findings suggest that changes in mGluR5 availability may be involved in the rapid antidepressant effect of sleep deprivation in MDD patients.

The mGluR5 are mostly expressed on post-synaptic membranes of neurons and astrocytes in cortico-limbic areas of the brain, including medial-prefrontal and orbito-frontal cortex, cingulate, striatum, amygdala and hippocampus (Gasparini et al., 2008a). They may be very well positioned to integrate and modulate the expression of established molecular markers of wakefulness and sleep. Indeed, mGluR5 interact either directly or indirectly with

Homer 1a, FMRP, BDNF, ADA, and ADORA2A. Convergent evidence obtained from studies in invertebrates, rodent models and humans strongly indicate that all these molecules play a causal role in sleep-wake regulation (Urade et al., 2003; Maret et al., 2007; Faraguna et al., 2008; Bushey et al., 2009; Bodenmann et al., 2011; Bushey et al., 2011).

For example, expression of the immediate early gene Homer 1a is increased after prolonged wakefulness (Maret et al., 2007). Homer 1a selectively uncouples mGluR5 from effector targets in the membrane of the post-synaptic density and attenuates the mGluR5-mediated rise in intra-cellular Ca^{2+} levels (Kammermeier and Worley, 2007). The interaction between Homer 1a and mGluR5 is necessary for mGluR5-dependent synaptic LTD (Ronesi and Huber, 2008) and may promote synaptic changes during sleep (Maret et al., 2007).

Not only Homer 1a but also FMRP interacts with mGluR5. A genetic defect in the X chromosome-linked human *FMR1* (Fragile X mental retardation 1) gene encoding FMRP gives rise to increased mGluR5 signaling and Fragile X syndrome (FXS), the most common form of inherited mental retardation and a leading cause of autism. Brain slices of *Fmr1* knock-out mice, an established model of FXS, show enhanced mGluR5-mediated synaptic LTD (Huber et al., 2002). *In vitro* as well as *in vivo*, these mice show prolonged spontaneous UP-states, which predominantly occur in SWS. This altered neocortical rhythmic activity is due to enhanced mGluR5 signaling (Hays et al., 2011). Sleep-wake regulation was studied in Drosophila mutants carrying *dFmr1* loss-of-function (amorphs) and gain-of-function (hypermorphs) mutations (Bushey et al., 2009). The *dFmr1* amorphs were ‘long

sleepers', whereas *dFmr1* hypermorphs were 'short sleepers'. A recent study further demonstrated that *dFmr1* is important for sleep-dependent synaptic normalization (Bushey et al., 2011).

Also interactions of mGluR5 with BDNF and adenosinergic neurotransmission may be important for sleep-wake regulation. Expression of BDNF in cerebral cortex is high during wakefulness, low during sleep, and increased after sleep deprivation (Cirelli and Tononi, 2000). Cortical injection of BDNF to awake animals promotes synaptic strength and enhances SWA in subsequent NREM sleep. This effect is reversible and opposite to the changes in local SWA after pharmacological inhibition of BDNF-tyrosine-kinase-β-receptor stimulation (Faraguna et al., 2008). Pharmacological activation of mGluR5 induces BDNF expression in rat cortical neurons and glia cells (Legutko et al., 2006; Viwatpinyo and Chongthammakun, 2009). It may, thus, be speculated that enhanced mGluR5-induced BDNF secretion after prolonged wakefulness contributes to the antidepressant response to sleep deprivation. Indeed, patients suffering from MDD typically exhibit reduced serum BDNF levels, which may normalize after antidepressant therapies (Nagahara and Tuszynski, 2011).

The facilitatory action of BDNF on hippocampal LTP requires adenosine A_{2A} receptor activation by endogenous adenosine (Fontinha et al., 2008). A primary role for adenosine and adenosine receptors in sleep regulation is well established in animals and humans (Basheer et al., 2004; Landolt, 2008b). In an approach similar to the present study, it was recently shown that sleep deprivation increases A_1 receptor binding in human brain (Elmenhorst et al.,

2007). Nevertheless, accumulating evidence indicates that also A_{2A} receptors contribute to sleep-wake regulation (Gallopin et al., 2005; Bodenmann et al., 2011). These receptors are co-localized with mGluR5, dopamine D_2, and NMDA receptors, and functionally interact *in vitro* and *in vivo* (Ferre et al., 2002a, 2002b; Tebano et al., 2005; Ciruela et al., 2006b, 2006a; D'Ascenzo et al., 2007). Consistent with our results, a PET study in humans revealed that endogenous $D_{2/3}$ receptor binding in the striatum was increased after sleep deprivation (Volkow et al., 2008). This increase was also associated with higher sleepiness and fatigue after sleep loss.

Because plasma samples were not available for calculating regional V_t, the cerebellum was used to calculate V_{norm}. mGluR5 receptor density in this region is unlikely to produce a specific binding signal with PET. Preclinical and clinical studies demonstrated that the cerebellum is suitable to quantify ^{11}C-ABP688 binding in the brain (Burger et al., 2010; Elmenhorst et al., 2010; Deschwanden et al., 2011). Another possible limitation of our study may be due to the fact that two different PET scanners were employed for examining the subjects. Because each volunteer was examined on the same scanner in sleep control and sleep deprivation conditions and a within-subject design was used, it is improbable that differences between PET scanners have biased the findings.

In conclusion, the current observations indicate that mGluR5 are involved in the effects of sleep deprivation. This receptor has been implicated in various central nervous system pathologies, and pharmacological agents targeting mGluR5 currently provide promising future treatments for psychiatric and

neurological disorders including schizophrenia, anxiety, FXS, substance abuse, and drug withdrawal (Gasparini et al., 2008a, 2008b; Niswender and Conn, 2010). Studies also suggest that mGluR5 agonists have antidepressant-like properties (Sanacora et al., 2008). Blockade of glutamatergic neurotransmission with the NMDA receptor antagonist ketamine and sleep deprivation therapy both rapidly reverse depression in a large subgroup of patients (Bunney and Bunney, 2011). Given the reduced mGluR5 density in depressed patients (Deschwanden et al., 2011) and the enhanced mGluR5 availability after prolonged wakefulness, it is tempting to hypothesize that mGluR5 are involved in the rapid mood-enhancing effects of sleep deprivation. Future research is needed to confirm this possible mechanism, and may lead to novel treatments of depression and other mental disorders which, according to the World Health Organization, will become the biggest health burden on society within the next 20 years.

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Disclosure / Conflict of interest

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Chapter 3

Challenging sleep-wake regulation in idiopathic hypersomnia: Evidence for impaired arousal

Katharina Hefti, MSc[1]; Ramin Khatami, MD[2]; Ursina Nadig, MSc[1]; Rositsa Poryazowa MD[2]; Birgit Högl, MD[3]; Claudio L. Bassetti MD[2], and Hans-Peter Landolt, PhD[1,4,5]

[1] Institute of Pharmacology & Toxicology, University of Zürich, Zürich, Switzerland

[2] Department of Neurology, University Hospital Zürich, Zürich, Switzerland

[3] Department of Neurology, Medical University, Innsbruck, Austria

[4] Neuroscience Center Zürich (ZNZ), University of Zürich & ETH Zürich, Zürich, Switzerland

[5] Zürich Center of Integrative Human Physiology (ZIHP), University of Zürich, Zürich, Switzerland

Manuscript in preparation

Abstract

Study Objectives: Patients with idiopathic hypersomnia (IH) suffer from excessive daytime sleepiness despite apparently undisturbed nocturnal sleep. The underlying pathophysiological mechanisms of IH are unknown. To investigate whether sleep homeostasis is altered in IH patients, we quantified sleep and waking EEG, as well as subjective sleepiness and neurobehavioral performance before, during, and after sleep deprivation.

Design: Adaptation and baseline nights were followed by 40 hours of prolonged wakefulness and a subsequent recovery night.

Setting: Sleep research laboratory.

Participants: Ten drug-naïve patients diagnosed with IH (6 women, 4 men) and 10 age- and sex-matched healthy controls completed the study.

Intervention: Prolonged wakefulness of 40 hours.

Measurements and Results: Visually scored sleep stages were similar in both groups. Prominently lower power in IH was present in α-, σ- and β-frequencies in non-rapid eye movement sleep (NREMS), and in the σ- and β-range in rapid-eye-movement sleep (REMS). Spectral analysis of the waking EEG revealed similar results. Frequencies higher than 11.5 Hz showed lower power in patients than in controls. In addition, neurobehavioral performance during prolonged wakefulness was worse in patients, and they rated themselves as being sleepier than controls. By contrast, salivary melatonin and cortisol concentration showed a nearly congruent evolution over time spent awake in both groups (n=5 per group).

Conclusion: The data suggest that the homeostatic and circadian sleep regulatory processes are intact in IH. The disease is characterized by impaired brain arousal as evidenced by reduced high-frequency EEG activity, elevated sleepiness, and reduced performance in the absence of a sleep disturbance.

Keywords: Idiopathic hypersomnia, sleep homeostasis, electroencephalogram, chronic fatigue, circadian.

Introduction

IDIOPATHIC HYPERSOMNIA (IH) IS A DISABLING DISEASE, DIFFICULT TO DIAGNOSE, RARELY STUDIED, AND THEREFORE POORLY UNDERSTOOD. THE TERM iHS HAS GENERALLY BEEN USED WITH CAU-tion due to the incomplete knowledge regarding the neuro-anatomical structures, neuro-physiological, and biochemical factors involved in the control of sleep and wakefulness (Guilleminault and Brooks, 2001). The International Classification of Sleep Disorders (AASM, 2005) presents clear rules to diagnose IH. The main factors considered for diagnosis are the duration of persisting excessive sleepiness, the time in bed sleeping, polysomnogram (to exclude other causes of daytime sleepiness), and the Multiple Sleep Latency Test (MSLT). The excess of sleep, which is the most apparent feature in IH, is best expressed in unrestricted conditions, such as during the weekend, on holidays, or in the sleep laboratory with sleep permitted ad-libitum. The average additional sleep seen in iHS patients compared to healthy controls has been reported to be around three hours (Vernet et al., 2010).

A widely accepted concept in sleep research is the two-process-model. It postulates that sleep is influenced by the interaction of the homeostatic process, which increases with time spent awake, and the endogenously guided circadian process (Borbély, 1982). Electroencephalographic (EEG) δ-activity (0.5-4.5 Hz), also referred to as slow-wave activity (SWA), represents a marker of the homeostatic process and is present in non-rapid eye movement sleep (NREMS). δ-activity has a peak power in the beginning of the sleep period and decreases

with time spent asleep. The amount of δ-activity can fluctuate and is a reflection of sleep need, as extended wakefulness or sleep deprivation will increase the amount of δ-activity seen during sleep. Another important characteristic feature of NREMS is the occurrence of sleep spindles (12-15 Hz), which are prominent in sleep stage 2. In contrast to δ-activity, sleep spindles show an increase in power density as sleep progresses. Furthermore, after prolonged hours of wakefulness, σ-activity is suppressed compared to the increase seen in δ-activity (Dijk et al., 1993). Rapid eye movement sleep (REMS) is the episode which follows a NREMS episode and ends a sleep cycle. With progression of the night, REMS episodes get longer. There might be two drives to enter REMS, namely, the homeostatic and the circadian drive. The homeostatic process, which is represented by δ-activity, rather suppresses REMS at the beginning of the night, and the circadian process leads to a favorable occurrence of REMS in the mornings (Aserinsky, 1969). After prolonged hours awake, the increased δ-activity suppresses REMS density in recovery sleep suggesting an inverse relationship between REMS density and sleep depth (Feinberg et al., 1987).

The interaction of the homeostatic process and the circadian process modulates not only sleep but also performance during the day (Borbély, 1982). The most disabling symptom IH patients suffer from is excessive daytime sleepiness (EDS). Therefore, the question arises whether the interaction of these two processes may be misaligned in IH. A frequently used protocol to study these processes is a period of prolonged hours awake. The homeostatic process can be estimated first by the difference in baseline and recovery night variables,

and second by an increase in specific EEG spectral parameters during the period awake. The circadian process can be assessed by repeated measurements of circadian biochemical markers such as melatonin (Cajochen et al., 2003), or cortisol in saliva (Van Cauter et al., 1996; Young et al., 2001). Measuring subjective sleepiness with questionnaires, or performance with computer-based cognitive tasks can also show the influence of both processes when monitored for more than 24 hours. The homeostatic process leads to an increase in sleepiness and a decrease in performance measures, whereas the circadian process leads to a partial improvement in performance the morning after one night of sleep deprivation.

These sleep variables have been implicated in IH. Based on previous studies, different hypotheses arose why IH could lead to cognitive difficulties, particularly in relation to alertness. Analysis of sleep cycles in one study showed lower SWA in IH patients during the first NREMS episodes stemming from a decreased amount of stage 3 and 4 sleep (Sforza et al., 2000), which could reflect a dysfunction in the build-up and/or decay in the homeostatic process. A sleep deprivation to discriminate between these possibilities was not performed. The amount and density of sleep spindles in stage 2 sleep may also play a role. Spindles were compared in a study of narcoleptic patients (NC), IH and healthy controls, with results showing that hypersomnolent patients had higher sleep spindle density than controls. Since the thalamus is involved in the generation of spindles and plays a role in the gatekeeping functions in NREMS, authors suggested that thalamic dysfunction may be involved in the pathophysiology of

IH (Bove et al., 1994). A further indication of a changed sleep physiology in IH was found by studying REMS in more detail (Vankova et al., 2001). It was observed that the number of eye movements per minute in REMS during the whole night was increased, and the increase to consecutive REMS episodes was higher in IH of polysymptomatic forms when compared to healthy controls. The authors suggested that the oculomotor activity is not only homeostatically regulated, but that a factor accounting for the pathophysiology of primary hypersomnia may be the central arousal system (Vankova et al., 2001). A study with a biochemical setting monitored melatonin in saliva. Saliva was sampled in regular intervals for 24 hours and compared across IH, NC and controls. A clear reduction in melatonin concentration was observed between controls and IH and, in addition, to a two-hour delay (Nevsimalova et al., 2000), indicating a possible deregulation in biochemical signaling and a delayed sleep phase, or longer circadian period. This assumption was supported by a study showing that patients with IH are more frequently evening types, and that most of them suffer from disabling sleep drunkenness in the mornings (Vernet et al., 2010). Another reason for the problems in vigilance may be an imbalance in neurotransmitter systems, in particular a decrease in dopaminergic transmission (Montplaisir et al., 1982; Saper et al., 2005).

The major aim of the study was to provide insighs into the pathophysiology of IH. We focused on sleep homeostasis by measuring sleep and the sleep EEG in baseline and recovery nights after sleep deprivation. During 40 hours of prolonged wakefulness, we collected additional measures,

including waking EEG recordings, questionnaires, performance testing, melatonin, and cortisol levels in saliva, to monitor the build-up of the homeostatic process in patients with IH and matched healthy controls.

Material & Methods

Subjects

The study procedures were approved by the cantonal ethic committee and carried out in accordance with the Declaration of Helsinki. All participants gave their written informed consent before participating in the study.

Ten IH patients (6 women, 4 men) with a mean age 32.5 ± 4.2 years participated in the study. None of them took medication during the study period. Patients were diagnosed by an experienced sleep neurologist according to the International Classification of Sleep Disorder (AASM, 1990, , 2005), and informed about the sleep-study. Interested patients were contacted by the responsible person of the study and received detailed information about all procedures. Controls were recruited from the general public of Zürich and its agglomeration. The healthy control subjects were individually matched with respect to age and sex (mean age: 33.4 ± 4.13 years). Weight and height were similar between the groups. All subjects were moderate alcohol and caffeine consumers. All controls reported to be good sleepers, a statement which was confirmed with a screening night prior to the study. In addition, they had no significant past or present psychiatric or neurological disorders, and were free of any medication intake or illicit drug abuse. Demographics for both patients and healthy controls are presented in Table 1.

Table 1. Demographics of idiopathic hypersomnia patients and their age- and sex-matched healthy controls.

	Idiopathic Hypersomnia	Controls	p=
Gender ratio	6 women, 4 men	6 women, 4 men	
Age	32.5 ± 4.2	33.4 ± 4.1	0.215
BMI	23.23 ± 0.9	23.67 ± 1.5	0.747
Daytime sleepiness	13.3 ± 1.3	7.6 ± 1.1	**0.016**
MSLT: SL SOREM	4.2 ± 0.4 0.2 ± 0.1	--	--
Trait anxiety	41.1 ± 2.7	35.2 ± 2.5	0.057
Habitual sleep duration [1]	8.4 ± 0.9	7.3 ± 0.2	0.403
Habitual sleep duration [2]	9.4 ± 0.6	8 ± 0.3	0.155
Caffeine consumption	2.8 ± 0.9	2.4 ± 0.6	0.951
Alcohol consumption	0.75 ± 0.2	2.7 ± 0.7	0.182

Values represent means ± SEM (n=10). Statistics represent paired two-tailed *t*-test.
BMI: Body mass index (kg/cm^2); Daytime sleepiness was measured with the Epworth Sleepiness Scale; MSLT: Multiple sleep latency test; SL: mean sleep latency across four measurements during one day; SOREM: sleep onset REMS episode; TAI: Trait Anxiety Inventory; Habitual sleep duration^1: reported habitual sleep length during working days; Habitual sleep duration^2: reported habitual sleep length during days off; caffeine consumption: caffeinated beverages/day; alcohol consumption: drinks/week. Caffeine and alcohol data were not available for three patients and one control.

Experimental Procedure

Three days prior to the study week all participants had to adhere to a regular sleep-wake schedule (8/16) and were not allowed taking naps during the day. In order to ensure compliance, wrist actigraphy was continuously measured during this period. The subjects were also requested to avoid caffeine and alcohol three day prior to, and during the experimental period.

Prior to the nights in the laboratory, saliva was taken for caffeine determination and breath alcohol was measured. The bedrooms were completely dark, temperature regulated and soundproof. The study design included an

adaptation night (AD) (23:30-07:30), a subsequent baseline night (BL) (23:30-07:30), a consecutive period of 40 hours of wakefulness and a recovery night (REC) (23:30-09:30). During prolonged wakefulness, subjects were under constant supervision by the research team to ensure wakefulness, and were allowed to read, play games, study, watch TV, and take a walk outside the sleep laboratory. Starting 15 minutes after lights-on following the baseline night, and subsequently at 3hour intervals, waking EEGs were recorded and subjective state and cognitive performance were measured. In addition, a saliva sample was obtained at 2-hour intervals in five patients and five controls throughout sleep deprivation (Salivette®, Sarstedt, 9475 Sevelen). The samples were stored at -20°C for later melatonin and cortisol determination.

One patient was unable to stay awake after 38 hours of prolonged wakefulness and initiated recovery night two hours earlier. For correct matching, the control subject went to bed after 38 hours as well. One IH patient was excluded from the EEG analyses due to a large deviation (> 2 SD) from the group mean. Because of a computer break-down lasting three hours, the EEG data of a recovery night in one IH patient were lost.

Data collection

Sleep and waking EEG

Continuous polysomnographic recordings were performed during all nights. Electrodes for the EEG were placed on the subjects' head according to the internationally used 10-20 system (Jasper, 1958). The surface electrodes

were placed at F3, F4, C3, C4, P3, P4, O1, and O2 and were referenced to A2. In addition, the electrooculogram (EOG), the electromyogram (EMG), and the electrocardiogram (ECG) were recorded by a polygraphic amplifier (PSA24; Braintronics, Almere, The Netherlands). The analog signals were conditioned by a high-pass filter (-3 dB at 0.16 Hz), a low-pass filter (-3 dB at 102 Hz and approximately -40 dB at 256 Hz), and a notch filter (50 Hz), digitized and transmitted via fiber-optic cables to a personal computer. Data were sampled with a frequency of 512 Hz, digitally filtered (EEG and EOG: low-pass FIR filter, -3 dB at 49 Hz; EMG: band-pass FIR filter, -3 dB at 15.6 and 54 Hz), and stored on a hard disk with a resolution of 128 Hz. Sleep stage scoring was performed visually, based on the C3A2 derivation, and using 20 second epochs according to standard rules (Rechtschaffen and Kales, 1968). The first 8 hours of the baseline and recovery night recordings were analyzed. The night was also subdivided into NREMS (S2+S3+S4) and REMS episodes. The first four NREMS episodes were included in the analyses of the evolution of EEG power across consecutive frequency bins, and the following frequency bands: δ (0.75-4.5 Hz), α (8-12 Hz), σ (11.25-15 Hz) and β (15-20 Hz).

EEG-raw data processing was performed with MATLAB (The MathWorks Inc, Natick, MA, USA) for sleep and waking EEG derived from the C3A2 derivation. To approximate a normal distribution, absolute EEG power densities were log-transformed before statistical testing.

EEG spectral power in sleep was computed for consecutive frequency bins (0.25 Hz resolution) in NREMS and REMS using the fast Fourier

transformation (FFT; Hanning window; average of five 4-s epochs; 0-20 Hz). Artifacts were identified by visual inspection, and a semiautomatic algorithm (moving average threshold) was used to separately exclude high- (20-40 Hz) and low- (0.75-4.5 Hz) frequency artifacts in each derivation.

The waking EEG was measured according to standardized criteria. The subject was alone in a soundproof room, the light intensity was <150 lux and he/she had his/her chin resting on a headrest to avoid movement artifacts. Via intercom, the subjects were asked to sit relaxed and to close their eyes for 3 minutes and open them for an additional 5 minutes recording. EEG, EOG, EMG and ECG signals were collected as described above. The waking EEG spectral power was computed by an FFT routine with some modifications, in contrast to the sleeping EEG (FFT, Hanning window, 50% overlapping 2 second epochs; 0.5 Hz resolution; 0-30 Hz). Artifacts in the waking EEG were visually identified and excluded. Mean spectra with eyes open are reported.

For time course analysis, the average of consecutive waking EEG sessions were calculated and expressed as a percentage of the average over all waking EEG recordings (Fig. 3). The last analysis comprised the determination of the α-peak during the period of prolonged hours awake. The absolute values were aligned to their individual α-peak ± SEM. Alpha-peak frequency was defined in each subject as the frequency bin with maximum power between 6 and 13 Hz. The α-peak frequency in patients was on average at 10.06 ± 0.51 Hz. Controls had their mean α-peak power at 10.39 ± 0.35 Hz. One subject in each group had no detectable α-peak and was excluded from this analysis.

Cognitive testing and biochemical markers

At 3-hour intervals subjective state was measured with the Karolinska sleepiness scale (KSS). In addition, neurobehavioral performance was measured using a 10minute psychomotor vigilance task (PVT, (Dinges and Powell, 1985)). Further information on the application of this task can be found in a previous publication (Bodenmann et al., 2009a). In addition, the current mood state was measured at 8 p.m. on the first day after 12 hours awake, and at 8 a.m. on the second day after 24 hours without sleep; this was accomplished with the state trait anxiety inventory (STAI, (Spielberger et al., 1970)).

The direct saliva melatonin RIA kit was used to measure melatonin by a double-antibody radio immunoassay, based on the Kennaway G280 anti-melatonin antibody, as in previous studies (Weber et al., 1997; Chellappa et al., 2012). Cortisol concentration was determined by a luminescence immunoassay according to the previously described protocol (Ditzen et al., 2009).

Statistical analyses

For statistical analysis, the SAS 9.1 software was used (SAS Institute, Cary, NC). Two factor mixed-model analyses of variance (ANOVA) were employed, including main factors ‘group’ (patients, controls), ‘condition’ (BL, REC), ‘NREMS episode’ (1, 2, 3, 4), and ‘time’ (time intervals during SD). The significance level was set at $\alpha < 0.05$ if not stated otherwise. To localize significant differences within a main factor, post-hoc paired two-tailed *t*-tests were performed.

Results

Idiopathic hypersomnia patients show similar sleep architecture, whereas the spectral analysis reveals differences to healthy controls

Visually scored sleep variables

Sleep variables in baseline and recovery sleep are summarized in Table 2. The two groups had similar sleep architecture in baseline sleep, and responded similarly to prolonged wakefulness in recovery sleep. The duration of stage 3 sleep was lower in IH in baseline and recovery nights. Sleep deprivation shortened sleep latency and increased sleep efficiency in the recovery night. Both groups showed a decrease in stage 1 sleep at the expense of increased stage 4 and SWS duration. As the only difference, the groups showed different responses to sleep deprivation in REMS latency. Controls showed a reduction in REMS latency in the recovery night when compared to the baseline night. This was not the case in patients with IH.

All-night EEG power spectra

Figures 1A and 1B present the whole night power spectra (0-20 Hz) in NREMS and REMS in baseline and recovery nights.

In NREMS there was a ‘group’ effect in the ranges 0-0.25 Hz, 3.5-10.25 Hz, 13.25-15 Hz and 17-20 Hz, and an effect of ‘condition’ in the range 0.5-9 Hz. Illustrating the patients’ values relative to controls and performing paired two-tailed *t*-tests revealed lower power in IH in the 6.5-9.5 Hz and 15.75-20 Hz ranges in baseline sleep and in the 7.5-9.5 Hz band in recovery sleep (Fig. 1C).

Analyzing frequency bands in NREMS revealed a clear response to prolonged hours awake by an increase in δ-power in recovery night, as compared to baseline night in both groups (Tab. 3).

An analysis of REMS power spectra revealed a prominent ‘group’ effect in the range 0-20 Hz, except the second bin, and a ‘condition’ effect in the range 1-2 Hz and 2.5-4.25 Hz. Plotting patient values relative to controls, as described previously, revealed lower power in IH in baseline (1.75-2 Hz, 3.25-4.25 Hz, 5-7.5 Hz and 11.25-20 Hz) and recovery night (1.5, 6, 10.25, 11.25, 15 Hz and 15.5-16 Hz and 16.75-20 Hz) compared to controls (Fig.1D). Sigmapower was lower in IH patients in both nights. Patients with IH showed decreased β-power in baseline and recovery nights. Detailed output of frequency band analysis of NREMS and REMS is given in Table 3.

Evolution of EEG power across NREMS episodes

The evolution of EEG activity over the first four NREMS episodes was analyzed separately for baseline and recovery nights. Baseline sleep analysis revealed an effect of ‘group’ between 2.75-12 Hz and 13-20 Hz, and an effect of ‘NREMS episode’ in 0-10.75 Hz frequencies (Fig. 2A and 2B). The same analysis, performed for recovery night, revealed a ‘group’ effect in 0-11.25 Hz, 13-17.5 Hz, 18.25-19 Hz and 19.75-20 Hz frequencies. A prominent effect of ‘NREM sleep episode’ was present between 0-11 Hz (Fig. 2C and 2D).

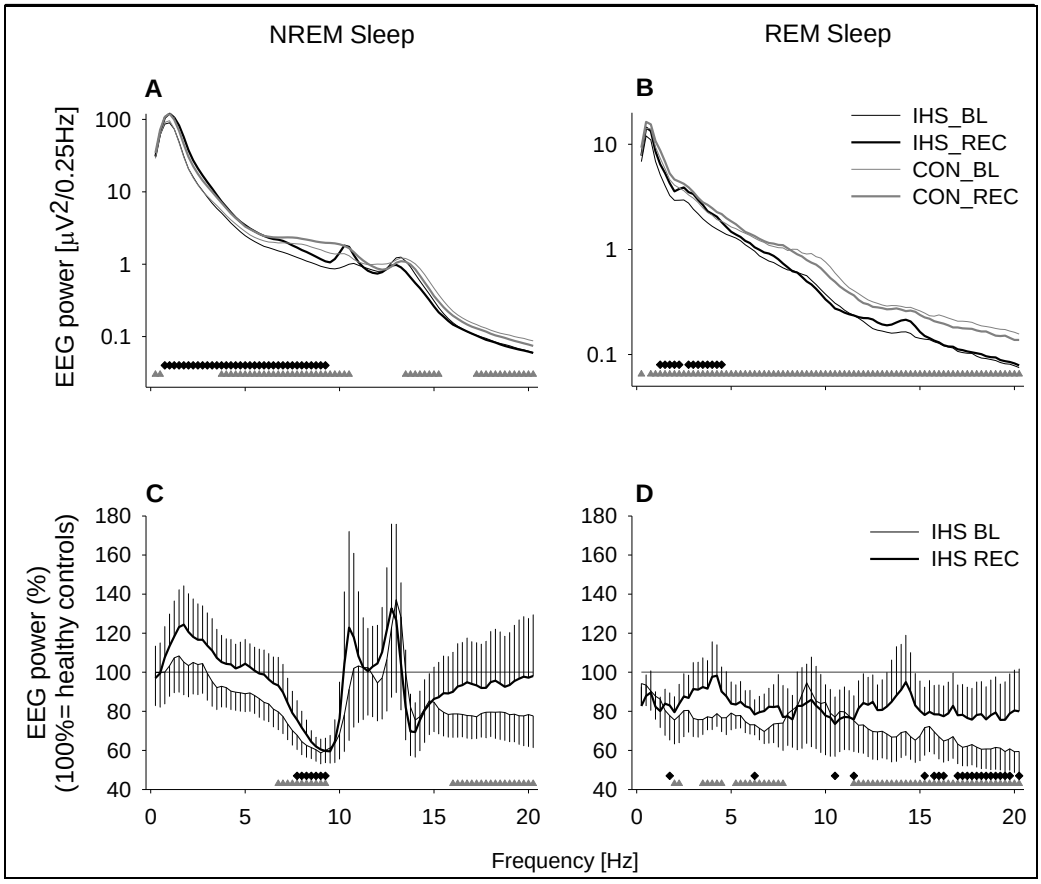

Figure 1. Sleep EEG data represent the first 8 hours after lights-off and derive from the C3A2 derivation. **A** & **B**: EEG power spectra in NREMS (left) and REMS (right) in baseline (BL) and recovery nights (REC) in 10 IH patients (black lines) and 10 controls (grey lines). Mixed model ANOVA, with factor 'group' and 'condition', revealed for NREMS a significant 'group' effect in the ranges 0-0.25 Hz, 3.5-10.25 Hz, 13.25-15 Hz and 17-20 Hz ($F_{1, 27} > 4.3$, $p < 0.05$), and a 'condition' effect in the range 0.5-9 Hz ($F_{1, 27} > 5.48$, $p < 0.05$). Mixed model ANOVA with factor 'group' and 'condition' in REMS revealed a highly significant 'group' effect from 0-20 Hz except the second bin ($F_{1, 24} > 4.79$, $p < 0.05$), a 'condition' effect in the range 1-2 Hz and 2.5-4.25 Hz ($F_{1, 24} > 4.3$, $p < 0.05$). Triangles represent a significant 'group' effect, and diamonds represent a significant effect of 'condition'. No bin revealed a significant 'group*condition' interaction.

C & **D**: EEG power values of IH are expressed as a percentage to the control values for NREMS and REMS in baseline- and recovery night. A paired two-tailed t-test is performed to compare the two groups. IH have lower power in NREMS in baseline night in the ranges 6.5-9.5 Hz and 15.75-20 Hz and in recovery night in the range 7.5-9.5 Hz. IH have lower power in REMS in baseline night in the ranges 1.75-2 Hz, 3.25-4.25 Hz, 5-7.5 Hz and 11.25-20 Hz, and in recovery night in the frequency bins at 1.5, 6, 10.25, 11.25, 15 Hz and the ranges 15.5-16 Hz and 16.75-20 Hz except the second last bin. Grey triangles represent lower power in baseline night iHS compared to healthy controls ($p< 0.05$) and in black diamonds in recovery night respectively.

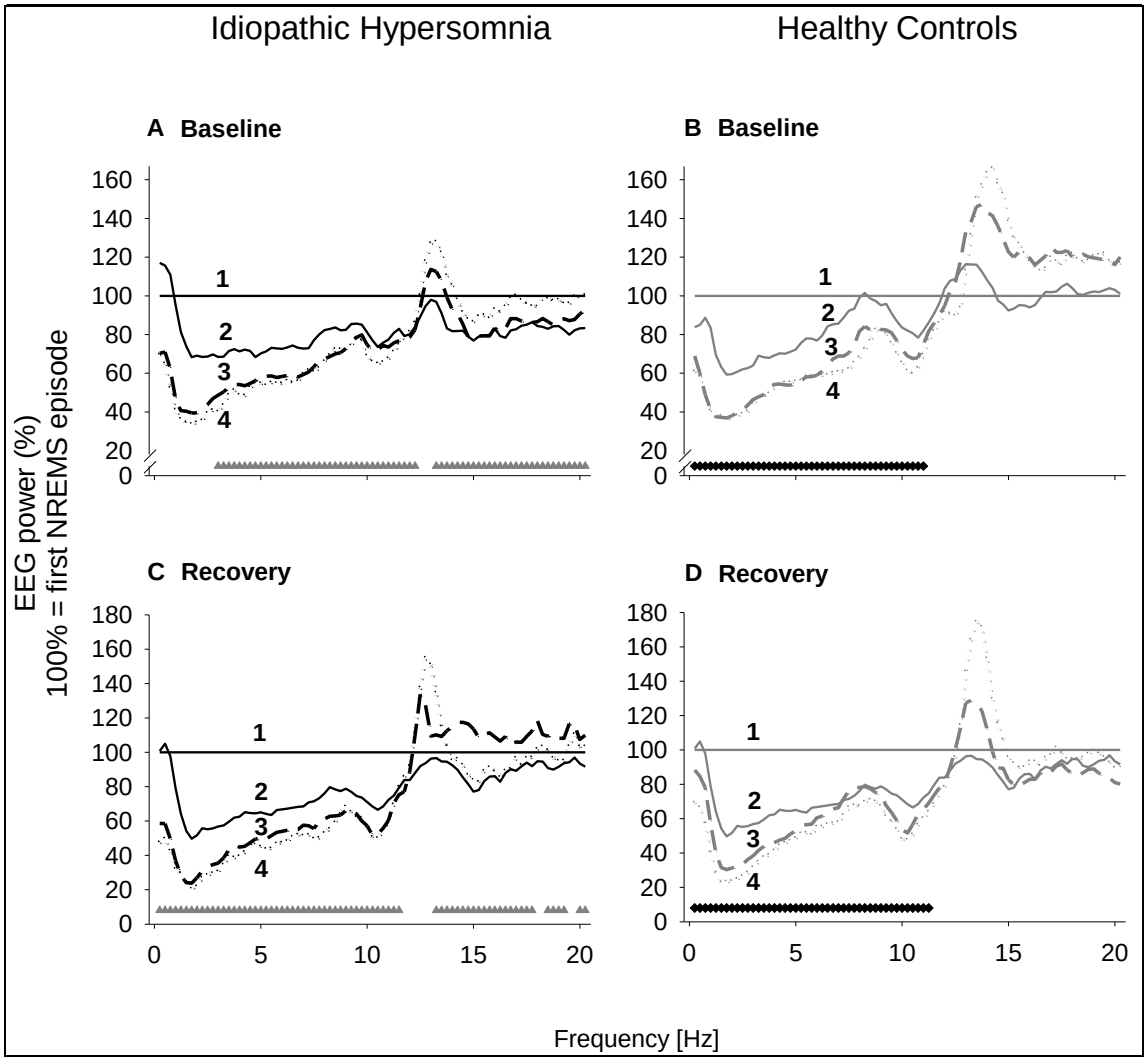

Figure 2. A - D: **Analysis of the first four NREMS episodes**. Cycles two, three and four are expressed as a percentage of the corresponding mean value of the first NREMS episode. Two-way ANOVA was performed for BL and REC separately with main factors 'group' and 'NREMS episode'. Analysis of BL night data revealed a significant 'group' effect in the ranges 2.75-12 Hz and 13-20 Hz ($F_{1, 56} > 4.63$, $p < 0.05$) and further a 'NREMS episode' effect in the ranges 0-10.75 Hz ($F_{3, 56} > 2.82$, $p < 0.05$). Analysis with REC night data revealed similar results. A significant 'group' effect in the ranges 0-11.25 Hz, 13-17.5 Hz, 18.25-19 Hz and 19.75-20 ($F_{1,54} > 4.08$, $p < 0.05$), and further a 'NREMS episode' effect in the ranges 0-11 Hz ($F_{3, 54} > 3.35$, $p < 0.05$). Triangles represent significant main effect of 'group' in BL and REC sleep, diamonds represent significant main effect of 'NREMS episode' in baseline and recovery night respectively.

Idiopathic hypersomnia patients have lower β-power during prolonged wakefulness

Power spectra during wakefulness

The power spectra (0-30 Hz), averaged over all 14 measurements during prolonged wakefulness, are visualized in Figure 3A. Paired two-tailed *t*-tests revealed in patients lower power in a broad range (11.5-30 Hz except bin at 15 Hz, $p < 0.05$).

Alignment to the α-peak revealed no difference in their position. Patients had a similar mean α-peak at 10.06 ± 0.51 Hz to controls α-peak at 10.39 ± 0.35 Hz ($p = 0.66$, paired two-tailed *t*-test).

Evolution of spectral power over 40 hours of wakefulness

To examine whether the evolution of EEG frequencies during prolonged wakefulness differs between the groups, the waking EEG was studied in more detail. Figures 3B and 3C visualize the effect of sleep loss on the waking EEG. Means of four consecutive waking EEG recordings were expressed as a percentage of the averaged EEG power over all 14 recordings. A large frequency range revealed statistical ‘group’ effect (2 Hz, 5.5-30 Hz). The factor ‘time’ was significant in δ-/θ-frequencies (0.5, 3.5-5.5 Hz) but no significant ‘group’ x ‘time’ interaction was present.

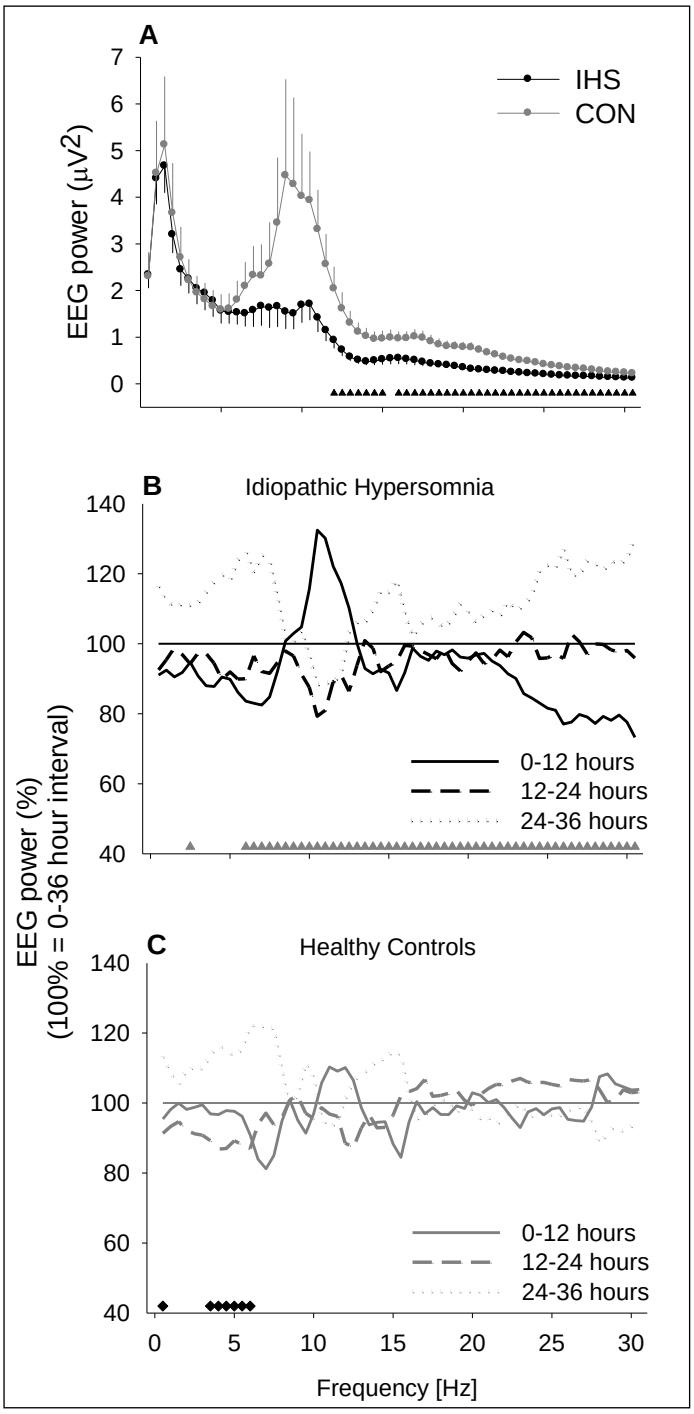

Figure 3. Waking EEGs were collected in 3-hour intervals during the 40 hours without sleep. Data with eyes open from the C3A2 derivation are presented.

A Mean absolute power density ± SEM is presented in IH and controls; all 14 measurements averaged. Paired two-tailed *t*-test revealed significantly lower power in IH between 11.5-30 Hz except for 15 Hz bin. Triangles represent $p< 0.05$. **B** & **C** Evolution of EEG power density in IH patients and healthy controls during prolonged hours awake. Mean over 4 consecutive measures were plotted relative to mean value over all wEEG. Two-way ANOVA with factor 'group' and 'time' and their interaction revealed significant main effect of 'group' in 2 Hz, 5.5-30 Hz ($F_{1.59} > 4.8$, $p< 0.05$), and an effect of 'time' in the first bin and in 3.5-5.5 Hz frequency bins ($F_{3,\ 59} > 3.06$, $p< 0.05$). Triangles in **B** represent 'group' effect and diamonds represent in **C** 'time' effect.

Reference line (100%) represent mean over all wEEG; solid line: mean over wEEG within 12 hours awake; dashed line: mean over wEEG during the time window 12-24 hours awake; dotted line: mean over wEEG during the time window 24-23 hours awake.

Idiopathic hypersomnia affects daytime functions

Subjective state

The evolution of subjective sleepiness was quantified with the Karolinska Sleepiness Scale (KSS). The values are represented in Figure 4A and show significantly higher sleepiness in patients than in controls throughout prolonged wakefulness ('Group' $F_{1, 224} = 66.03$, $p < 0.0001$).

The state anxiety inventory revealed a significant effect of 'time' ('time' $F_{1, 27} = 12.08$, $p < 0.002$). Patients had a score of 39.7 ± 2.5 at 8 p.m. the first evening, and 44.1 ± 3.3 at 8 a.m. after one night without sleep. Controls showed a score of 33.0 ± 3.1 at day one and of 44.1 ± 3.8 on day two. Paired two-tailed *t*-test revealed a significantly higher anxiety score on the first evening in patients with IH compared to healthy controls ($p = 0.04$).

Psychomotor vigilance task (PVT)

Performance on the PVT shows detrimental effects in healthy volunteers after prolonged wakefulness (Durmer and Dinges, 2005). Here, three PVT variables were analyzed (Fig. 4B-D). The fastest 10^{th} percentile of reaction times (RTs) showed that patients were able to perform RTs equally fast as controls, even after prolonged hours awake ('Group' $F_{1, 29.8} = 1.56$, $p > 0.22$). By contrast, the slowest 10^{th} percentile of RTs showed slower speed in patients throughout the whole period ('Group' $F_{1, 30.8} = 16.7$, $p < 0.001$). In addition, the number of lapses (RT> 500 ms) was higher in patients than in controls ('Group' $F_{1, 38.4} = 9.0$, $p < 0.005$).

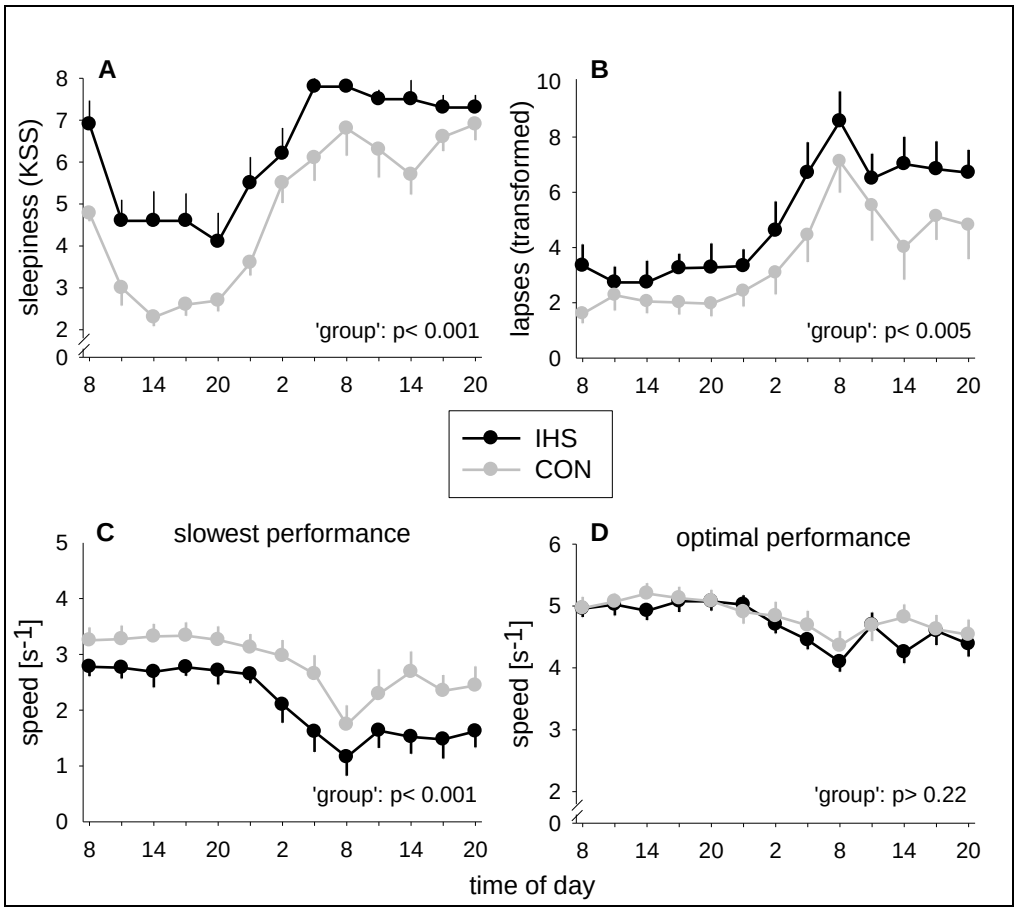

Figure 4. Effect of 40 hours prolonged wakefulness on subjective and objective measures. Data were collected at 3-hour intervals during sleep deprivation. All four measures show a circadian modulation and increased homeostatic sleep pressure on the second half of the period without sleep. **A** Time course of sleepiness quantified with the Karolinska Sleepiness Scale (KSS). Throughout the whole period IH felt sleepier than controls. 'Group' $F_{1, 86.1}$ = 73.14, p< 0.001; 'time' $F_{12, 126}$ = 21.48, p< 0.001; 'group*time' $F_{12, 149}$ = 0.93, p= 0.5148. **B** Time course of transformed lapses ($\sqrt{x}+\sqrt{x+1}$; RT > 500 ms) on the PVT. Similar to the slowest RTs, IH had more lapses than controls during prolonged hours awake. 'Group' $F_{1, 38.4}$ = 9.0, p< 0.001; 'time' $F_{12, 140}$ = 8.18, p< 0.001; 'group*time' $F_{12, 135}$= 0.57, p= 0.8638. **C** Time course of the slowest 10th percentile of RTs on the PVT. During the whole period iHS had slower RTs than controls. 'Group' $F_{1, 30.8}$ = 16.70, p< 0.001; 'time' $F_{12, 149}$ = 7.2, p< 0.001; 'group*time' $F_{12, 138}$ = 0.66, p=0.7854. **D** Time course of the fastest 10th percentile of reaction times (RT) on the psychomotor vigilance task (PVT, task duration: 10 minutes). The graph shows a nearly congruent evolution of the fastest RT between the groups. Statistics show no significant difference between iHS and controls 'group' $F_{1, 29.8}$ = 1.56, p> 0.22; 'time' $F_{12, 147}$ = 6.24, p< 0.001; 'group*time' $F_{12, 136}$ = 0.1.89, p= 0.0404. Values represent means ± SEM in 10 IH patients (black symbols) and 10 controls (grey symbols).

Idiopathic hypersomnia does not influence endocrinological markers of circadian clock output

Melatonin

The evolution of salivary melatonin concentration was nearly congruent across the groups (Fig. 5A). Averaged melatonin concentrations during the night (8 p.m. to 8 a.m.) were 2.65 ± 1.44 pg/ml in patients with IH and 3.08 ± 1.58 pg/ml in controls ($p < 0.37$). All absolute values were in the normal physiological range (Burgess and Fogg, 2008).

Cortisol

The evolution in salivary cortisol concentration revealed no difference between the two groups over 40 hours of prolonged wakefulness (Fig. 5B). The maximal concentration was seen in both groups within a time window of 2 hours (7 to 9 a.m.). The absolute concentrations (mean over 24h: iHS: 8.6 ± 1.69 nmol/ml; CON: 9.02 ± 2.47 nmol/ml) lay in both groups in the standard range of healthy individuals and did not differ across the groups ($p < 0.63$) (Aardal and Holm, 1995).

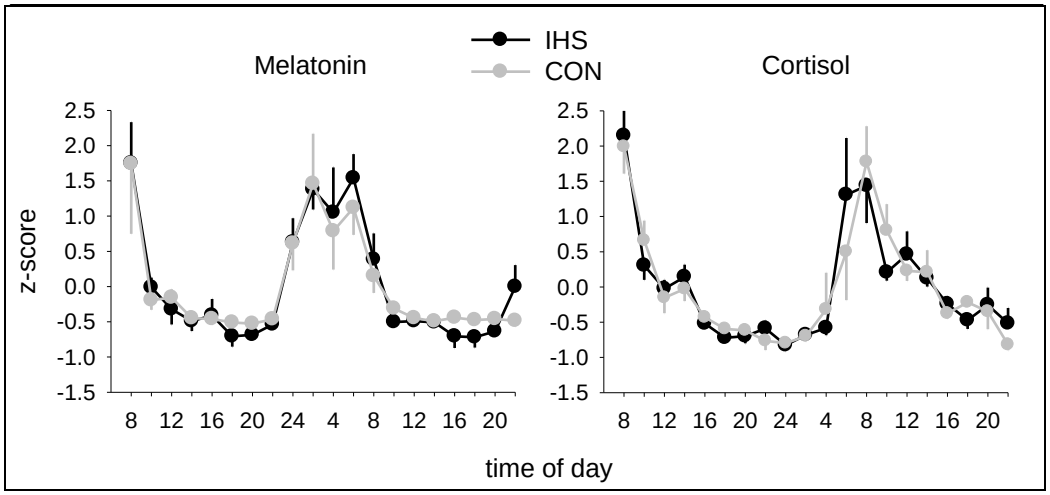

Figure 5. Salivary melatonin and cortisol profiles in saliva during prolonged wakefulness. Saliva was sampled in 2-hour intervals during the sleep deprivation period. The evolutions of both biochemical variables show normal circadian modulation in diseased and healthy subjects.

Saliva was available in 5 matched subjects of each group. Values represent z-scores per group ± SEM; Black symbols represent idiopathic hypersomnia patients (IH), grey symbols represent control subject (CON).

Mixed model ANOVA of melatonin data, with factor 'group' and 'time', revealed a highly significant effect of 'time' but none for 'group' and their interaction. 'Group' $F_{1,156} = 0.21$, p= 0.6505; 'time' $F_{19, 156} = 4.99$, p< 0.0001; 'group*time' $F_{19, 156} = 0.24$, p= 0.9997.

The same results arouse for cortisol analysis. 'Group' $F_{1,156} = 0.15$, p= 0.7017; 'time' $F_{19, 156} = 9.78$, p< 0.0001; 'group*time' $F_{19, 156} = 0.56$, p=0.9263.

Table 2. Visually scored sleep variables for baseline and recovery sleep in idiopathic hypersomnia patients and controls.

Variable	**Idiopathic Hypersomnia**		**Controls**		**'Group'**	**'Condition'**	**'Group' x 'Condition'**
	Baseline	Recovery	Baseline	Recovery	$F_{1,26}$ (p)	$F_{1,26}$ (p)	$F_{1,26}$ (p)
WASO	4.6 ± 1.4	6.6 ± 3.5	11.1 ± 4.8	6.4 ± 3.1	2.19 (0.15)	0.47 (0.50)	1.84 (0.19)
Stage 1	30.0 ± 7.9	17.2 ± 4.4 †	23.0 ± 4.1	12.7 ± 2.7 †	1.78 (0.19)	**9.62 (0.0046)**	0.21 (0.65)
Stage 2	207.3 ± 9.5	194.8 ± 10.2	205.6 ± 9.0	195.3 ± 12.5	0.01 (0.94)	2.43 (0.13)	0.08 (0.77)
Stage 3	30.7 ± 3.2	32.7 ± 3.8	39.6 ± 5.4	41.7 ± 4.9	**6.11 (0.0203)**	0.56 (0.46)	0.03 (0.89)
Stage 4	64.5 ± 11.7	101.3 ± 13.1 †	58.6 ± 10.1	103.2 ± 14.7 †	0.27 (0.61)	**39.26 (0.0001)**	0.13 (0.72)
SWS	95.2 ± 13.5	134.0 ± 12.1 †	98.2 ± 12.5	144.9 ± 17.3 †	0.43 (0.52)	**37.96 (0.0001)**	0.06 (0.81)
REM	111.6 ± 4.6	107.0 ± 5.5	113.6 ± 7.0	104.7 ± 4.9	0.00 (0.97)	1.44 (0.24)	0.15 (0.70)
MT	14.1 ± 2.0	11.0 ± 0.9	14.9 ± 1.7	10.7 ± 1.6 †	0.03 (0.86)	**6.33 (0.0182)**	0.13 (0.72)
TST	444.1 ± 6.5	453.0 ± 4.5	440.4 ± 5.7	457.6 ± 2.5 †	0.00 (0.95)	**14.42 (0.0008)**	1.29 (0.27)
SL	17.1 ± 5.2	9.4 ± 2.7 ‡	12.7 ± 4.6	5.3 ± 1.5	1.73 (0.2)	**5.18 (0.0312)**	0.00 (0.98)
RL	55.4 ± 7.1	66.5 ± 10.4	63.4 ± 2.9	55.6 ± 2.2 †	0.72 (0.4)	0.77 (0.39)	**4.70 (0.0393)**
SEFF	92.5 ± 1.4	94.4 ± 1.4	91.8 ± 1.2	95.3 ± 0.5 †	0.01 (0.94)	**14.21 (0.0008)**	1.27 (0.27)

Values represent means ± SEM in minutes (except sleep efficiency in %) of nine idiopathic hypersomnia patients and controls. The first 8 hours (= 480 minutes) after lights-off were analyzed. † $p < 0.03$ (paired, two-tailed *t*-test within group); ‡ $p < 0.05$ (paired, two-tailed *t*-test between group). Patients with IH show similar amount of sleep variables as their healthy controls. The interaction of REM sleep arose because one subject started with sleep onset REM. Exclusion of this subject revealed the following statistical values: 'group' 2.00 (0.17); 'condition' 0.16 (0.79); 'group' x 'condition' 3.13 (0.09).

WASO: Wake After Sleep Onset; SWS: Slow Wave Sleep (Stage3 and 4) REM: Rapid Eye Movement; MT: Movement Time; TST: Total Sleep Time; SL: Sleep Latency (first occurrence of stage 2); RL: REM Latency; SEFF: Sleep Efficiency (percentage of TST per 480 minutes). Due to computer breakdown, the data of one patient's recovery night are missing.

Table 3. Power in NREM and REM sleep EEG in distinct frequency bands.

	Idiopathic Hypersomnia		**Controls**		**'Group'**	**'Condition'**	**'Group' x 'Condition'**
	Baseline	Recovery	Baseline	Recovery	$F_{1, 27}$ (P)	$F_{1, 27}$ (P)	$F_{1, 27}$ (P)
NREMS							
Delta	365.5 ± 68.4	552.1 ± 109.1 †	328.2 ± 77.0	444.3 ± 112.3 †	1.35 (0.2547)	7.08 (**0.0129)**	0.30 (0.5886)
Alpha	16.4 ± 2.6	20.3 ± 4.1	20.7 ± 2.6	25.0 ± 3.9	2.70 (0.1117)	1.09 (0.3057)	0.02 (0.8778)
Sigma	12.3 ± 2.9	10.3 ± 2.0	13.9 ± 2,2	12.2 ± 2.1	2.87 (0.1020)	1.38 (0.2496)	0.00 (0.9543)
Beta	2.3 ± 0.1	2.2 ± 0.2	3.1 ± 0.3	2.7 ± 0.3	5.56 (**0.0259)**	1.52 (0.2290)	0.24 (0.6253)
REMS							
Delta	47.6 ± 8.6	59.9 ± 12.2	56.5 ± 10.9	65.1 ± 12.8	1.90 (0.1797)	3.13 (0.0883)	0.21 (0.6538)
Alpha	7.6 ± 1.3	6.8 ± 0.8	10.5 ± 2.1	9.1 ± 1.6	1.84 (0.1868)	0.24 (0.6260)	0.01 (0.9277)
Sigma	3.1 ± 0.3	3.4 ± 0.4	4.5 ± 0.6	4.2 ± 0.5	4.53 (**0.0402)**	0.02 (0.8809)	0.28 (0.5994)
Beta	2.2 ± 0.1 ‡	2.2 ± 0.2	3.7 ± 0.6	3.2 ± 0.5	7.46 (**0.0110)**	0.09 (0.7642)	0.26 (0.6147)

Values represent whole night NREM and REM power spectra (μV^2/0.25 Hz). Values show mean power ± SEM for a two way ANOVA with main factor 'group' and 'condition' and their interaction for ten IH patients and healthy controls. † $p < 0.03$ (paired, two-tailed *t*-test within group); ‡ $p < 0.05$ (paired, two-tailed *t*-test between groups).

Delta (0.75-4.5 Hz), Alpha (8-12 Hz), Sigma (11.25-15 Hz), Beta (15-20 Hz).

Discussion

This is the first study performing a controlled sleep deprivation protocol in IH patients to challenge sleep-wake regulation. It is furthermore the first time in IH to report electroencephalographic data across night and day in the same individuals, in comparison to individually age- and sex-matched controls.

We found no profound qualitative and quantitative differences in baseline- and recovery sleep in visually scored sleep variables. With this finding, we support the conclusion of a recent study that disrupted nocturnal sleep is infrequently observed in patients with IH (Takei et al., 2012). Nevertheless, in all vigilant states patients with IH had lower β-EEG power than controls. Intriguingly, there was no difference present in whole night spectral power between the groups, independent of night. Differences were present in higher frequencies, including θ-, α-, σ- and β-ranges to various degrees. IH is a disease with distinct characteristics when compared to other sleep disorders. For example, patients with narcolepsy have a deficiency in the hypocretin system of the lateral hypothalamus (Nishino et al., 2000; Kanbayashi et al., 2002; Anderson et al., 2007). Furthermore, these patients experience disturbed NREMS-REMS patterns and strong sleep fragmentation, which underlies their non restorative sleep (Khatami et al., 2007; Khatami et al., 2008). Daytime sleepiness is the only common symptom in patients with IH and patients with narcolepsy. Another sleep disorder is insomnia which is defined by long sleep latency, frequent nocturnal awakenings, prolonged periods of wakefulness during a sleep episode, or

frequent transient arousals (Roth, 2007). It was shown that patients with insomnia have increased β-activity in NREM sleep (Perlis et al., 2001; Bonnet and Arand, 2010). These findings suggest that insomnia is a disorder in which the arousal system is hyperactive (Bonnet and Arand, 2010). It is possible that the simultaneous, inappropriate activity of wake- and sleep-promoting systems in patients with insomnia underlie their inability to fall asleep. In contrast, patients with IH have a long and undisturbed nocturnal sleep. Despite this fact, patients with IH are sleepy during the day. Our study showed reduced β-power in patients with IH when compared to healthy controls.

Similarly to patients with IH, patients with chronic fatigue syndrome (CFS) also suffer from unexplained persisting daytime sleepiness, and show significantly lower power in σ- and β-frequencies in sleep stage 2, SWS and REMS when compared to healthy controls (Decker et al., 2009). Although sleep architecture in patients with CFS does not differ from healthy controls (Majer et al., 2007), it was proposed that impaired sleep homeostasis contributes to the symptoms of CFS, because patients showed a blunted SWA response compared to controls to a 4-hour delay in bedtime (Armitage et al., 2007). Our study revealed no evidence that sleep homeostasis is impaired in patients with IH.

Regarding the subjective data, IH patients had higher state anxiety than controls at 8 p.m., and a similar score at 8 a.m. in the morning after the night without sleep. In contrast, controls had a significantly lower score at baseline compared to IH; this score increased from the first to the second day to the same

level as IH. This result is in accordance with a study reporting higher anxiety (Hospital Anxiety and Depression rating scale, HAD) in IH than in controls. In the same study, IH and controls had to fill in a 5 point tiredness score (Vernet et al., 2010). The patients were on a higher level during the whole day, a fact we could also show with the KSS.

Performance on PVT is a measure of the sustained attention (Drummond et al., 2005). This task was not been previously applied to patients with IH. The results show reduced performance in the patient group. In general, it can be stated that sleep deprivation impaired performance in IH and controls in a similar manner, but on different levels.

Biochemical markers showed similar evolution in both groups. Thus, we conclude that the circadian regulation of melatonin and cortisol secretion is intact in patients with IH. However, our data do not permit the definite conclusion that patients with IH have normal circadian rhythms, because we did not perform an experiment in the absence of any time information.

In comparison with previous studies perfomed in patients with IH, our results show different findings. First, we did not find decreased SWA in IH compared to controls, as suggested by Sforza et al. (2000). Thus, our data to not support the idea of a changed build-up of the homeostatic process. We cannot rule out that the effect they observed was confounded by extended sleep duration in the patients prior to the sleep study, because sleep duration was not controlled for. Second, in contrast to Bové et al. (1994), we did not find a

difference in σ-power between patients with IH and healthy controls. In our study, we did not find a significant difference in σ-power, if anything σ-power tended to be lower in IH patients compared to controls. Again, the difference could have been caused by different sleep duration prior to the study night, since Bové et al. did not control for sleep length before their experimental recordings (Bove et al., 1994). This is an important aspect, because reduced sleep pressure such as after naps increases σ-power, whereas sleep deprivation decreases σ-power (Dijk et al., 1993; Knoblauch et al., 2003). Thus, differences in prior sleep duration could have biased the previous results. Third, REMS latency showed a difference between the groups. Patients with IH showed a prolongation of the REMS latency from baseline to recovery night, weheras the opposite was found in the controls. The REMS latency was significantly longer in patients with IH when compared to healthy controls. Regarding the previously mentioned study, which found an increased REM density in REMS in IH compared to controls (Vankova et al., 2001), we did not analyse this variable and can, therefore, not compare the respective findings. Forth, salivary melatonin and cortisol concentrations revealed a normal secretion pattern over 24 hours and no difference between the groups. Therefore, we find no evidence to claim that IH have a misaligned circadian rhythm. A possible reason for the discrepancy to the study, in which a delayed secretion of both hormones was found (Nevsimalova et al., 2000) could lie in the control of habitual sleep episodes. Nevsimalova et al.did not control for regular wake-sleep schedule, nor for time of day when the

subjects went to bed. The observed delay in hormone secretion could be biased by late awakenings and late bed times.

A possible imbalance in neurotransmitter systems, including the ascending arousal system (AAS), could underlie problems in vigilance. The reciprocal activities and interactions of wake-active and sleep-active cell groups determine the alternation between wakefulness and sleep (Saper et al., 2005). We assume that cholinergic cells, originating in the upper pons and the basal forebrain, and monoaminergic cells, originating from the brainstem, discharge mainly during behavioral arousal (Hallanger et al., 1987; Saper et al., 2011) to maintain wakefulness. The sleep promoting neurons originate mainly in the ventral preoptic area (VLPO), and are active during sleep by inhibiting, via GABA-ergic neurons, wake promoting regions such as the locus coeruleus (LC), tuberomammillary nucleus (TMN), and raphe nucleus (RN) (Saper et al., 2005). Reduced activity of these nuclei could underlie the increased sleepiness in patients with IH. Another explanation could be that the VLPO is hyperactive and attenuates the wake-active cell groups during wakefulness. Taken together, the EDS can either occur because of a decreased function of the AAS, or due to a hyperactivity of the VLPO. This hyperactivity in turn, suppresses AAS regions such as the LC, MTN and RN so strongly that a state transition to wake only occurs with effort which leads to sleep inertia in IH.

We suggest that the phenotype of these dysregulated mechanisms in IH is EDS. To treat patients with EDS, a combination of behavioral and

pharmacological therapies are used. Today, mainly two drugs are administered to IH, namely, antidepressants and modafinil (Schwartz, 2004; Ballon and Feifel, 2006). Currently used antidepressants inhibit reuptake of monoamines, which are the most prevalent neurotransmitter in the AAS and innervate the cerebral cortex for maintaining arousal. The site of action of modafinil is still unclear, but it leads to a reduction in EDS in IH (Gerrard and Malcolm, 2007). Authors suggest that modafinil blocks the reuptake of noradrenalin by the noradrenergic terminals on sleep-promoting neurons from the VLPO (Gallopin et al., 2004). An imaging study suggested that administration of modafinil increased extracellular DA and occupied DA transporters (Volkow et al., 2009). Therefore, it is likely that modafinil promotes dopaminergic neurotransmission.

Conclusions

The present study elaborated on the effect of 40 hours of wakefulness on sleep and waking EEG, and performance in patients with IH and matched controls. The major findings of this project are: (1) patients with IH have similar sleep variables as controls in baseline and recovery sleep; (2) patients with IH have a decreased high frequency EEG power, independently of vigilance state; (3) patients with IH have reduced performance during prolonged wakefulness; and (4) patients with IH have no change in salivary melatonin and cortisol concentration when compared to healthy controls.

With our findings we have no evidence for impaired sleep homeostatic and circadian processes in patients with IH. We rather conclude that these patients

suffer from deficient brain arousal that could be a sign of reduced monoaminergic activity.

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Chapter 4

4.1 Discussion

In this thesis, the effect of extended wakefulness was investigated with respect to changes in receptor availability in healthy subjects (project I), and in view of objective markers in spectral power in sleep and waking EEG in patients with IH (project II).

In the following sections, our findings will be discussed and contextualized within current research.

Metabotropic glutamate receptor subtype 5 – mGluR5

In the first project, we quantified the availability of mGluR5 in the human brain using positron emission tomography (PET). The ligand ^{11}C-ABP$_{688}$ binds selectively to this receptor. Twenty-two healthy men participated in a two week, randomized crossover study. In one week, participants performed a PET scan after 9h of wakefulness, whereas in the other week the PET scan took place after they were kept awake for one night (i.e., after 32h of wakefulness).

We found that cerebral mGluR5 availability has increased in the healthy human brain after a single night without sleep, indicating that mGluR5 contributes to the effects of sleep deprivation. Out of 13 volumes of interest (VOI) we analyzed, six regions showed a Bonferroni-corrected significant increase in receptor availability after prolonged wakefulness. These regions were the anterior cingulate cortex,

insula, medial temporal lobe, parahippocampal gyrus, striatum, and amygdala. This increase was positively correlated with the subjectively rated increased sleepiness.

Forty years ago, it was postulated that the homeostatic process underlying sleep regulation builds up during wakefulness and dissipates during sleep (Borbély, 1982). However, the molecular and cellular mechanisms of the homeostatic process are unknown. The recently postulated synaptic homeostasis hypothesis is a conceptual framework that takes the two-process model of sleep-wake regulation, and the cellular properties into account (Tononi and Cirelli, 2006). According to this hypothesis, the number of synapses is upscaled during wakefulness and downscaled during sleep, in a use-dependant manner. A study in rats showed that in cortex and hippocampus GluR1-containing AMPA receptor (AMPAR) levels were high during wakefulness, and low during sleep (Vyazovskiy et al., 2008). Similarly, the phosphorylation of AMPAR was higher after wakefulness than after sleep in these rats. The authors postulate that with this up- and down-scaling, which goes in line with the phosphorylation and dephosphorylation of receptors in accordance to wakefulness and sleep, an overall balance of synaptic strength is preserved (Vyazovskiy et al., 2008). Our findings are consistent with the assumption of the synaptic homeostasis hypothesis during wakefulness. We observed that extended waking increased the global mGluR5 availability in the human brain. Similar findings were published by another group that studied adenosine A1 receptor (A_1AR) binding

potential after sleep deprivation (Elmenhorst et al., 2007). These authors found that prolonged hours awake increased the binding potential of A_1AR. They suggested that A_1AR expression contributes to homeostatic sleep regulation. Our findings may also reflect synaptic potentiation: an increase in receptor density would lead to an increase in the strength of signal transmission between dendrites and axons. To corraborate a role for mGluR5 in sleep homeostasis, mGluR5 availability needs to be quantified after recovery sleep to show that it returns to the baseline level.

A sleep deprivation study in three inbred mouse strains showed that Homer1a is overexpressed in a genotype-dependant manner. This overexpression was observed in the cortex, caudate and hippocampus (Maret et al., 2007). This finding is of interest, since different studies showed a close interaction between mGluR5 and Homer1a (Ango et al., 2000; Kammermeier and Worley, 2007; Bertaso et al., 2010; Tronson et al., 2010). The main consequence of mGluR5 activation is the release of calcium from the postsynaptic intracellular calcium storage. Homer1a acts as an adaptor; it is an activity-dependent negative domain that regulates the scaffolding and signaling capabilities of the long Homer forms, and reduces glutamate-induced intracellular calcium release via the uncoupling of inositoltriphosphat (IP_3) (Xiao et al., 2000; Kammermeier and Worley, 2007; Worley et al., 2007). It was shown that Homer1a expression alters mGluR signaling, even though large changes in mGluR organization are not seen (Kammermeier and Worley, 2007). Therefore, the plastic change of mGluR5 may

represent a viable mechanism to finetune synaptic strength on a short-term basis. Homer1a was proposed as a core molecular marker of sleep loss (Maret et al., 2007). Homer1a prevents the formation of the second messenger IP_3 after activation of mGluR5 and reduces calcium release from intracellular storage. Moreover mGluR5 is involved in LTD. The increase in receptor availability after prolonged wakefulness could be a signal for a stronger downscaling of synapses during recovery sleep. However, to corroborate the underlying mechanism of these dynamic processes, further studies are needed.

Various nervous system pathologies have been implicated with deregulated amounts of mGluR5. This receptor family provides a promising target to treat in the future psychiatric and neurological disorders, including schizophrenia, anxiety, fragile X syndrome, substance abuse, and drug withdrawal (Gasparini et al., 2008a; Niswender and Conn, 2010; Nagahara and Tuszynski, 2011). One study in depressed patients showed decreased mGluR5 availability in various cortical and subcortical brain regions when compared to healthy controls (Deschwanden et al., 2011). We found in healthy subjects increased availability of mGluR5 after prolonged wakefulness; this indicates that this receptor may be involved in the potent antidepressant effect of ‘wake therapy’ in patients with major depressive disorder. It is well established that sleep deprivation provides robust and rapid full clinical relief in many patients suffering from major depression (Bunney and Bunney, 2011). A possible underlying mechanism could be that increased receptor availability after sleep loss can lead to increased or

prolonged neuronal signaling. In contrast to available antidepressants, which take 2 to 3 weeks to exert an effect, our findings are of interest because of the fast response of mGluR5 availability.

Another point to discuss is the relatively small, 4% to 5% increase in mGluR5 availability from sleep control to sleep deprived condition. Although small, this increase is highly significant and reliable because of intraindividual normalization to cerebellum mGluR5 levels. We used this method to control for between-subject variability, to reduce the standard deviation, and to increase the signal-to-noise ratio. Because the mGluR5 is a G-coupled receptor, it amplifies downstream signaling in an exponential manner (Ross, 1989). Therefore, a change of roughly 5% in G-coupled receptor availability, in 21 healthy volunteers, is not only significant from a statistical point of view, but most likely also demonstrates a relevant physiological difference.

The first evidence for increased cerebral mGluR5 availability after extended wakefulness was revealed with the present, in vivo molecular imaging study in healthy human volunteers. After a single night without sleep, binding of the non-competitive ligand ^{11}C-ABP$_{688}$ was significantly increased on a global level, and in distinct brain regions which were previously shown to reflect physiological changes after sleep deprivation (Dang-Vu et al., 2010).

Idiopathic hypersomnia - IH

In the second project, we performed the first sleep deprivation study in patients with IH and their age- and sex-matched controls. With this protocol, we could

examine the response of patients with IH to prolonged hours of wakefulness on subjective and objective measures in comparison to healthy controls.

We found similar sleep architecture in IH compared to controls. Power spectral analysis revealed the characteristic δ-decay during the first four NREMS episodes in both patients and controls, indicating normal function of the homeostatic process. High frequencies were significantly reduced in patients compared to controls. This reduced power was present in all three vigilance states, and possibly represents a characteristic marker in the EEG of patients with IH. During wakefulness, lower EEG power was present in all analyzed frequency bands. The evolution of melatonin and cortisol concentration in saliva during the 40h wakefulness showed a nearly congruent shape with no difference in timing and amplitude.

Previous studies in patients with IH showed reduced SWA and an increased sleep spindle density when compared to healthy controls, while the time course of the homeostatic decay during sleep was not affected (Bove et al., 1994; Sforza et al., 2000). Our results did not corroborate these findings. By contrast, we found a similar amount of SWA in baseline and recovery sleep in IH and controls. A further result, which is not in line with previous findings, is the secretion rate of melatonin and cortisol. The published study found a reduced melatonin and a delayed cortisol secretion in IH compared to controls (Nevsimalova et al., 2000). In contrast, we found similar melatonin and cortisol concentration in saliva in

patients with IH compared to controls. A possible confounder is the habitual sleep-wake time, which was not controlled for by these the authors. A delayed cortisol secretion could be a consequence of late awakenings. A study performed in insomnia patients showed that they had decreased melatonin secretion and increased cortisol secretion at night (Bonnet and Arand, 2010). The only valid experiment to observe circadian shifts would be a free running study where the subjects would live for several days without any light and time cues. Such data could provide a valid statement about changes in circadian rhythmicity. With respect to patients with IH, another imaging study with insomnia patients revealed that they had higher global cerebral glucose metabolism during wakefulness and sleep, and further, a smaller decline in relative metabolism from wakefulness to sleep state in wake promoting regions. Therefore, they conclude that the higher brain metabolism could be associated with the difficulty of falling asleep. This could be interpreted as a failure in the arousal system to decline from the transition of wakefulness to sleep (Nofzinger et al., 2004). A future imaging study in patients with IH could help to clarify whether they have a reduced brain metabolism, and therefore would lead to EDS. An EEG study in CFS patients showed lower power in all frequencies analyzed (Decker et al., 2009). By analyzing similar frequency bands in our study, we observed the same findings. We found that our patients had, in all analyzed frequency bands, lower power compared to controls. These results, spurred the idea of a diminished arousal system to keep these patients awake.

Sleep-wake regulation is influenced by neurotransmitter systems and the AAS. It is possible that patients with IH have a lower neurotransmitter tone, which would lead to an inappropriate suppression of the VLPO by the wake promoting regions of the AAS, namely, the LC, TMN, and RN (Saper et al., 2005).

The VLPO neurons, which are GABA-ergic, project to the same wake-promoting regions and inhibit them. We found sleep variables in IH patients that are similar to controls, and suggest that the sleep GABA-promoting system is intact. Since the regions of the ventral pathway of the AAS are monoaminergic, it is probable that IH patients have a dysregulated monoaminergic neurotransmission.

The question arises whether a link between endogenous DA and sleepiness exists. Mean firing rates of dopaminergic neurons in the substantia nigra and the ventral tegmental area have no profound change over the sleep-wake cycle in rats (Miller et al., 1983). Since others have found a rhythmicity of DA concentration in hypothalamus, medial prefrontal cortex, and nucleus accumbens (Eriksson et al., 1980; Lena et al., 2005), it is rather believed that the DA secretion pattern underlies a circadian modulation. Five DA G-protein coupled receptor families are separated and are grouped in D_1-like family or D_2-like familiy. D_1-like family, containing D_1R and D_5R, activates adenylate cyclase (AC) which leads to an increase in intracellular cyclic adenosine monophosphate (cAMP). In contrast, the D_2-like family, containing D_2, D_3 and $D_{4,}$ leads to an inhibition of the formation of cAMP by inhibiting the enzym AC (Neves et al., 2002). DA receptors are mainly located in the cerebral cortex, the limbic system, hypothalamus, and striatum. A study, published 30 years ago, showed

decreased DA concentration in cerebrospinal fluid in patients with IH (Montplaisir et al., 1982). Furthermore, a human PET study found a decreased tracer binding to $D_{2/3}$ receptors after sleep loss (Volkow et al., 2008). The authors interpreted this finding to reflect increased endogenous DA levels after prolonged wakefulness. The changes were associated with higher sleepiness and fatigue after sleep loss. Nevertheless, a follow-up study of the same research group showed that endogenous DA was not increased but D_2 receptor density was decreased after sleep loss in healthy volunteers (Volkow et al., 2012). By contrast, preliminary data in seven IH patients suggest increased DA receptor availability by using PET imaging. This increase was present in the putamen and nucleus caudatus (Bassetti et al., 2009). This finding may be consistent with reduced endogenous DA signalling.

Dopamine-enhancing drugs increase wakefulness by blocking DA transporters. Modafinil is suggested to stimulate the release of DA from the presynapse leading to an enhanced DA tone, and a prolonged DA mediated synaptic transmission (Nishino and Mignot, 2005). Others state that Modafinil blocks the reuptake of noradrenalin (NA) by the noradrenergic terminals on sleep-promoting neurons from the VLPO (Gallopin et al., 2004). It is most likely that DA and NA play a role in promoting both cortical activation and behavioral arousal of waking and, thus, are useful in treating hypersomnolence (Nishino and Mignot, 1997).

Little is known about objective measures in patients with IH. To our knowledge, we performed the first sleep deprivation study in IH patients with additional

daytime recording in the same subjects. The spectral power in high frequencies was reduced in patients, independent of vigilant state. We suggest that rather than sleep, it is the waking functions which are diminished in IH patients.

mGluR5 and IH

Taking the findings of the two studies together, two questions may be considered for future studies:

1) Do patients with IH have higher mGluR5 levels than controls under basal condition?

2) Does a potential competitive and selective mGluR5 antagonist decrease sleepiness in healthy controls and IH patients?

With these two aspects, we would obtain a better understanding of whether subjective sleepiness and performance are associated with high basal mGluR5 availability, and whether an antagonist could decrease the excessive sleepiness in patients by modulating the receptors downstream signaling. Whether an antagonist administered to healthy subjects can lead to an even more increased performance, is subject of further interest.

4.2 Concluding remarks

From an evolutionary point of view, sleep is a universal phenomenon. All species studied to date show a sleep-like phenotype. It is thought that sleep in some way evolved to serve restoration. Because sleep is seen in all studied animals, it is suggested that it serves a crucial physiological function which, however, is still unknown.

References

Aardal E, Holm AC (1995) Cortisol in saliva--reference ranges and relation to cortisol in serum. European Journal of Clinical Chemistry and Clinical Biochemistry 33:927-932.

AASM AAoSM (1990) International classification of sleep disorders: Diagnostic and coding manual, 1st Edition. Rochester, Minnesota: American Sleep Disorders Association.

AASM AAoSM (2005) THE INTERNATION CLASSIFICATION OF SLEEP DISORDERS Diagnostic and Coding Manual, 2nd Edition. Westchester, IL: Westbrook Corporate Center.

Achermann P (2004) The two-process model of sleep regulation revisited. Aviation, Space, and Environmental Medicine 75:A37-43.

Achermann P (2009) EEG Analysis Applied to Sleep. Epileptologie 26:28-33.

Achermann P, Borbély AA (2003) Mathematical models of sleep regulation. Frontiers in Bioscience 8:683-693.

Achermann P, Borbély A (2011) Sleep homeostasis and models of sleep regulation. In: Principles and Practice of Sleep Medicine, 5 Edition (Kryger M, Roth T, Dement W, eds), pp 431-444. St. Louis, MI: Elsevier Saunders.

Adamantidis AR, Zhang F, Aravanis AM, Deisseroth K, de Lecea L (2007) Neural substrates of awakening probed with optogenetic control of hypocretin neurons. Nature 450:420-424.

Aeschbach D, Dijk DJ, Borbély AA (1997) Dynamics of EEG spindle frequency activity during extended sleep in humans: relationship to slow-wave activity and time of day. Brain Research 748:131-136.

Agnew HW, Jr., Webb WB, Williams RL (1967) Comparison of stage four and 1-rem sleep deprivation. Perceptual and Motor Skills 24:851-858.

Akerstedt T, Gillberg M (1990) Subjective and objective sleepiness in the active individual. International Journal of Neuroscience 52:29-37.

Aldrich MS, Chervin RD, Malow BA (1997) Value of the multiple sleep latency test (MSLT) for the diagnosis of narcolepsy. Sleep 20:620-629.

Ambrosius U, Lietzenmaier S, Wehrle R, Wichniak A, Kalus S, Winkelmann J, Bettecken T, Holsboer F, Yassouridis A, Friess E (2008) Heritability of sleep electroencephalogram. Biological Psychiatry 64:344-348.

Ametamey SM, Kessler LJ, Honer M, Wyss MT, Buck A, Hintermann S, Auberson YP, Gasparini F, Schubiger PA (2006) Radiosynthesis and preclinical evaluation of 11C-ABP688 as a probe for imaging the metabotropic glutamate receptor subtype 5. Journal of Nuclear Medicine 47:698-705.

Ametamey SM, Treyer V, Streffer J, Wyss MT, Schmidt M, Blagoev M, Hintermann S, Auberson Y, Gasparini F, Fischer UC, Buck A (2007) Human PET studies of metabotropic glutamate receptor subtype 5 with 11C-ABP688. Journal of Nuclear Medicine 48:247-252.

Anderson KN, Pilsworth S, Sharples LD, Smith IE, Shneerson JM (2007) Idiopathic hypersomnia: a study of 77 cases. Sleep 30:1274-1281.

Ango F, Pin JP, Tu JC, Xiao B, Worley PF, Bockaert J, Fagni L (2000) Dendritic and axonal targeting of type 5 metabotropic glutamate receptor is regulated by homer1 proteins and neuronal excitation. Journal of Neuroscience 20:8710-8716.

Anwyl R (2009) Metabotropic glutamate receptor-dependent long-term potentiation. Neuropharmacology 56:735-740.

Armitage R, Landis C, Hoffmann R, Lentz M, Watson NF, Goldberg J, Buchwald D (2007) The impact of a 4-hour sleep delay on slow wave activity in twins discordant for chronic fatigue syndrome. Sleep 30:657-662.

Aserinsky E (1969) The maximal capacity for sleep: Rapid eye movement density as an index of sleep satiety. Biological Psychiatry 1:147-159.

Aserinsky E, Kleitman N (1953) Regularly occurring periods of eye motility, and concomitant phenomena, during sleep. Science 118:273-274.

Aston-Jones G, Bloom FE (1981) Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. Journal of Neuroscience 1:876-886.

Aton SJ, Seibt J, Dumoulin M, Jha SK, Steinmetz N, Coleman T, Naidoo N, Frank MG (2009) Mechanisms of sleep-dependent consolidation of cortical plasticity. Neuron 61:454-466.

Aurlien H, Gjerde IO, Aarseth JH, Eldoen G, Karlsen B, Skeidsvoll H, Gilhus NE (2004) EEG background activity described by a large computerized database. Clinical Neurophysiology 115:665-673.

Ayala JE, Chen Y, Banko JL, Sheffler DJ, Williams R, Telk AN, Watson NL, Xiang Z, Zhang Y, Jones PJ, Lindsley CW, Olive MF, Conn PJ (2009) mGluR5 positive allosteric modulators facilitate both hippocampal LTP and LTD and enhance spatial learning. Neuropsychopharmacology 34:2057-2071.

Bachmann V, Klein C, Bodenmann S, Schafer N, Berger W, Brugger P, Landolt HP (2012) The BDNF Val66Met Polymorphism Modulates Sleep Intensity: EEG Frequency- and State-Specificity. Sleep 35:335-344.

Baglioni C, Spiegelhalder K, Lombardo C, Riemann D (2010) Sleep and emotions: a focus on insomnia. Sleep Medicine Reviews 14:227-238.

Ballon JS, Feifel D (2006) A systematic review of modafinil: Potential clinical uses and mechanisms of action. Journal of Clinical Psychiatry 67:554-566.

Barker AJ, Ullian EM (2010) Astrocytes and synaptic plasticity. Neuroscientist 16:40-50.

Basheer R, Strecker RE, Thakkar MM, McCarley RW (2004) Adenosine and sleep-wake regulation. Progress in Neurobiology 73:379-396.

Bassell GJ, Gross C (2008) Reducing glutamate signaling pays off in fragile X. Nature Medicine 14:249-250.

Bassetti C, Aldrich MS (1997) Idiopathic hypersomnia. A series of 42 patients. Brain 120 (Pt 8):1423-1435.

Bassetti C, Gugger M (2000) [Hypersomnia-etiology, clinic, diagnosis and therapy of excessive sleepiness]. Therapeutische Umschau 57:421-429.

Bassetti C, Khatami R, Poryazova R (2009) Idiopathic hypersomnia: a dopaminergic disorder. Sleep 32:248-249.

Bastuji H, Jouvet M (1988) Successful treatment of idiopathic hypersomnia and narcolepsy with modafinil. Progress in Neuropsychopharmacology & Biological Psychiatry 12:695-700.
Bear MF, Huber KM, Warren ST (2004) The mGluR theory of fragile X mental retardation. Trends in Neuroscience 27:370-377.
Beck AT, Erbaugh J, Ward CH, Mock J, Mendelson M (1961) An inventory for measuring depression. Archives of General Psychiatry 4:561-571.
Benington JH, Heller HC (1995) Restoration of brain energy metabolism as the function of sleep. Progress in Neurobiology 45:347-360.
Berger (1929) Über das elektroencephalogramm des Menschen. Archiv für Psychiatrie 87:527-570.
Bertaso F, Roussignol G, Worley P, Bockaert J, Fagni L, Ango F (2010) Homer1a-dependent crosstalk between NMDA and metabotropic glutamate receptors in mouse neurons. PLoS One 5:e9755.
Billiard M (2007) Diagnosis of narcolepsy and idiopathic hypersomnia. An update based on the International classification of sleep disorders, 2nd edition. Sleep Medicine Reviews 11:377-388.
Billiard M (2011) Idiopathic hypersomnia. In: Sleepiness: Causes, Consequences and Treatment (Michael J. Thorphy MB, ed), pp page 126 - 133: Cambridge University Press.
Billiard M, Dauvilliers Y (2001) Idiopathic Hypersomnia. Sleep Medicine Reviews 5:349-358.
Blethyn KL, Hughes SW, Toth TI, Cope DW, Crunelli V (2006) Neuronal basis of the slow (<1 Hz) oscillation in neurons of the nucleus reticularis thalami in vitro. Journal of Neuroscience 26:2474-2486.
Bliss TV, Gardner-Medwin AR (1973a) Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. The Journal of Physiology 232:357-374.
Bliss TV, Lomo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. the Journal of Physiology 232:331-356.
Bliss TV, Gardner-Medwin AR (1973b) Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. J Physiol 232:357-374.
Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31-39.
Bloch KE, Schoch OD, Zhang JN, Russi EW (1999) German version of the Epworth Sleepiness Scale. Respiration 66:440-447.
Bodenmann S, Xu S, Luhmann U, Arand M, Berger W, Jung H, Landolt H (2009a) Pharmacogenetics of Modafinil After Sleep Loss: Catechol-O-Methyltransferase Genotype Modulates Waking Functions But Not Recovery Sleep. Clinical Pharmacology & Therapeutics 85:296-304.
Bodenmann S, Hohoff C, Freitag C, Deckert J, Retey JV, Bachmann V, Landolt HP (2011) Polymorphisms of ADORA2A modulate psychomotor vigilance and the effects of caffeine on neurobehavioural performance and sleep EEG after sleep deprivation. Br J Pharmacol 165:1904-1913.

Bodenmann S, Rusterholz T, Durr R, Stoll C, Bachmann V, Geissler E, Jaggi-Schwarz K, Landolt HP (2009b) The functional Val158Met polymorphism of COMT predicts interindividual differences in brain alpha oscillations in young men. Journal of Neuroscience 29:10855-10862.

Bonnet MH, Arand DA (1994) Sleep onset: Normal and abnormal processes. . Washington, DC, US: American Psychological Associatin.

Bonnet MH, Arand DL (2010) Hyperarousal and insomnia: state of the science. Sleep Medicine Reviews 14:9-15.

Borbély AA (1982) A two process model of sleep regulation. Human Neurobiology 1:195-204.

Borbély AA (2001) From slow waves to sleep homeostasis: new perspectives. Archives Italiennes de Biologie 139:53-61.

Borbély AA, Achermann P (2005) Sleep homeostasis and models of sleep regulation. In: Principles and Practice of Sleep Medicine, 4th Edition (Kryger MH, Roth T, Dement WC, eds), pp 405-417. Philadelphia, PA: Elsevier Saunders.

Bove A, Culebras A, Moore JT, Westlake RE (1994) Relationship between sleep spindles and hypersomnia. Sleep 17:449-455.

Braun AR, Balkin TJ, Wesenten NJ, Carson RE, Varga M, Baldwin P, Selbie S, Belenky G, Herscovitch P (1997) Regional cerebral blood flow throughout the sleep-wake cycle. An H2(15)O PET study. Brain 120 (Pt 7):1173-1197.

Bunney BG, Bunney WE (2011) Rapid-acting antidepressant strategies: mechanisms of action. The International Journal of Neuropsychopharmacology:1-19.

Burger C, Deschwanden A, Ametamey S, Johayem A, Mancosu B, Wyss M, Hasler G, Buck A (2010) Evaluation of a bolus/infusion protocol for 11C-ABP688, a PET tracer for mGluR5. Nuclear Medicine and Biology 37:845-851.

Burgess HJ, Fogg LF (2008) Individual differences in the amount and timing of salivary melatonin secretion. PLoS One 3:e3055.

Bushey D, Tononi G, Cirelli C (2009) The Drosophila fragile X mental retardation gene regulates sleep need. Journal of Neuroscience 29:1948-1961.

Bushey D, Tononi G, Cirelli C (2011) Sleep and synaptic homeostasis: structural evidence in Drosophila. Science 332:1576-1581.

Cai Z, Schools GP, Kimelberg HK (2000) Metabotropic glutamate receptors in acutely isolated hippocampal astrocytes: developmental changes of mGluR5 mRNA and functional expression. Glia 29:70-80.

Cajochen C, Foy R, Dijk DJ (1999) Frontal predominance of a relative increase in sleep delta and theta EEG activity after sleep loss in humans. Sleep Research Online 2:65-69.

Cajochen C, Krauchi K, Wirz-Justice A (2003) Role of melatonin in the regulation of human circadian rhythms and sleep. Journal of Neuroendocrinology 15:432-437.

Cajochen C, Brunner DP, Kräuchi K, Graw P, Wirz-Justice A (1995) Power density in theta/alpha frequencies of the waking EEG progressively increases during sustained wakefulness. Sleep 18:890-894.

Chellappa SL, Viola AU, Schmidt C, Bachmann V, Gabel V, Maire M, Reichert CF, Valomon A, Gotz T, Landolt HP, Cajochen C (2012) Human melatonin and alerting response to blue-enriched light depend on a polymorphism in the clock gene PER3. Journal of Clinical Endocrinology and Metabolism 97:E433-437.

Chen LY, Rex CS, Casale MS, Gall CM, Lynch G (2007) Changes in synaptic morphology accompany actin signaling during LTP. Journal of Neuroscience 27:5363-5372.

Cirelli C, Tononi G (2000) Differential expression of plasticity-related genes in waking and sleep and their regulation by the noradrenergic system. Journal of Neuroscience 20:9187-9194.

Ciruela F, Casado V, Rodrigues RJ, Lujan R, Burgueno J, Canals M, Borycz J, Rebola N, Goldberg SR, Mallol J, Cortes A, Canela EI, Lopez-Gimenez JF, Milligan G, Lluis C, Cunha RA, Ferre S, Franco R (2006a) Presynaptic control of striatal glutamatergic neurotransmission by adenosine A1-A2A receptor heteromers. J Neurosci 26:2080-2087.

Ciruela F, Casado V, Rodrigues RJ, Lujan R, Burgueno J, Canals M, Borycz J, Rebola N, Goldberg SR, Mallol J, Cortes A, Canela EI, Lopez-Gimenez JF, Milligan G, Lluis C, Cunha RA, Ferre S, Franco R (2006b) Presynaptic control of striatal glutamatergic neurotransmission by adenosine A1-A2A receptor heteromers. Journal of Neuroscience 26:2080-2087.

Citri A, Malenka RC (2008) Synaptic plasticity: multiple forms, functions, and mechanisms. Neuropsychopharmacology 33:18-41.

Cobb SR, Bulters DO, Davies CH (2000) Coincident activation of mGluRs and mAChRs imposes theta frequency patterning on synchronised network activity in the hippocampal CA3 region. Neuropharmacology 39:1933-1942.

Cooley WJ, Tukey JW (1965) An algorithm for the Machine Calculation of Complex Fourier Series. Mathematics of Computation 19:297-301.

D'Ascenzo M, Fellin T, Terunuma M, Revilla-Sanchez R, Meaney DF, Auberson YP, Moss SJ, Haydon PG (2007) mGluR5 stimulates gliotransmission in the nucleus accumbens. Proceedings of the National Academy of Science USA 104:1995-2000.

Daan S, Beersma DG, Borbely AA (1984) Timing of human sleep: recovery process gated by a circadian pacemaker. American Journal of Physiology 246:R161-183.

Dang-Vu TT, Schabus M, Desseilles M, Sterpenich V, Bonjean M, Maquet P (2010) Functional neuroimaging insights into the physiology of human sleep. Sleep 33:1589-1603.

Dang-Vu TT, Schabus M, Desseilles M, Albouy G, Boly M, Darsaud A, Gais S, Rauchs G, Sterpenich V, Vandewalle G, Carrier J, Moonen G, Balteau E, Degueldre C, Luxen A, Phillips C, Maquet P (2008) Spontaneous neural activity during human slow wave sleep. Proceedings of the National Academy of Science USA 105:15160-15165.

Danker-Hopfe H, Anderer P, Zeitlhofer J, Boeck M, Dorn H, Gruber G, Heller E, Loretz E, Moser D, Parapatics S, Saletu B, Schmidt A, Dorffner G (2009) Interrater reliability for sleep scoring according to the Rechtschaffen & Kales and the new AASM standard. Journal of Sleep Research 18:74-84.

Datta S, Spoley EE, Patterson EH (2001) Microinjection of glutamate into the pedunculopontine tegmentum induces REM sleep and wakefulness in the rat. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 280:R752-759.

Dauvilliers Y, Baumann CR, Carlander B, Bischof M, Blatter T, Lecendreux M, Maly F, Besset A, Touchon J, Billiard M, Tafti M, Bassetti CL (2003) CSF hypocretin-1 levels in narcolepsy, Kleine-Levin syndrome, and other hypersomnias and neurological conditions. Journal of Neurology, Neurosurgery & Psychiatry 74:1667-1673.

De Gennaro L, Marzano C, Fratello F, Moroni F, Pellicciari MC, Ferlazzo F, Costa S, Couyoumdjian A, Curcio G, Sforza E, Malafosse A, Finelli L, Pasqualetti P, Ferrara M, Bertini M, Rossini PM (2008) The EEG fingerprint of sleep is genetically determined: a twin study. Annals of Neurology:in press (DOI 10.1002/ana.21434).

Decker MJ, Tabassum H, Lin JM, Reeves WC (2009) Electroencephalographic correlates of Chronic Fatigue Syndrome. Behavioral and Brain Functions 5:43.

Deese J (1959) On the prediction of occurrence of particular verbal intrusions in immediate recall. Journal of Experimental Psychology 58:17-22.

DeLorenzo C, Kumar JS, Mann JJ, Parsey RV (2012) In vivo variation in metabotropic glutamate receptor subtype 5 binding using positron emission tomography and [11C]ABP688. Journal of Cerebral Blood Flow & Metabolism 31:2169-2180.

Dement W (1960) The effect of dream deprivation. Science 131:1705-1707.

Dement W, Kleitman N (1957) Cyclic variations in EEG during sleep and their relation to eye movements, body motility, and dreaming. EEG Clinical Neurophysiology 9:673-690.

Dement WC (1965) Recent studies on the biological role of rapid eye movement sleep. American Journal of Psychiatry 122:404-408.

Deschwanden A, Karolewicz B, Feyissa AM, Treyer V, Ametamey SM, Johayem A, Burger C, Auberson YP, Sovago J, Stockmeier CA, Buck A, Hasler G (2011) Reduced Metabotropic Glutamate Receptor 5 Density in Major Depression Determined by [11C]ABP688 PET and Postmortem Study. American Journal of Psychiatry 168:727-734.

Dijk DJ, Hayes B, Czeisler CA (1993) Dynamics of electroencephalographic sleep spindles and slow wave activity in men: effect of sleep deprivation. Brain Research 626:190-199.

Dinges DF, Powell JW (1985) Microcomputer analyses of performance on a portable, simple visual reaction task during sustained operations. Behavior Research Methods, Instruments, & Computers 17:652-655.

Ditzen B, Schaer M, Gabriel B, Bodenmann G, Ehlert U, Heinrichs M (2009) Intranasal oxytocin increases positive communication and reduces cortisol levels during couple conflict. Biological Psychiatry 65:728-731.

Dolen G, Osterweil E, Rao BS, Smith GB, Auerbach BD, Chattarji S, Bear MF (2007) Correction of fragile X syndrome in mice. Neuron 56:955-962.

Dressendorfer RA, Kirschbaum C, Rohde W, Stahl F, Strasburger CJ (1992) Synthesis of a cortisol-biotin conjugate and evaluation as a tracer in an immunoassay for salivary cortisol measurement. The Journal of Steroid Biochemistry and Molecular Biology 43:683-692.

Drummond SP, Bischoff-Grethe A, Dinges DF, Ayalon L, Mednick SC, Meloy MJ (2005) The neural basis of the psychomotor vigilance task. Sleep 28:1059-1068.

Durmer JS, Dinges DF (2005) Neurocognitive consequences of sleep deprivation. Seminars in Neurology 25:117-129.

Ehrengruber MU, Kato A, Inokuchi K, Hennou S (2004) Homer/Vesl proteins and their roles in CNS neurons. Molecular Neurobiology 29:213-227.

Elmenhorst D, Minuzzi L, Aliaga A, Rowley J, Massarweh G, Diksic M, Bauer A, Rosa-Neto P (2010) In vivo and in vitro validation of reference tissue models for the mGluR(5) ligand [(11)C]ABP688. Journal of Cerebral Blood Flow & Metabolism 30:1538-1549.

Elmenhorst D, Meyer PT, Winz OH, Matusch A, Ermert J, Coenen HH, Basheer R, Haas HL, Zilles K, Bauer A (2007) Sleep deprivation increases A(1) adenosine receptor binding in the human brain: A positron emission tomography study. Journal of Neuroscience 27:2410-2415.

Eriksson E, Eden, Modigh K, Haggendal J (1980) Ultradian rhythm in rat hypothalamic dopamine levels. Journal of Neural Transmission 48:305-310.

Fagni L, Chavis P, Ango F, Bockaert J (2000) Complex interactions between mGluRs, intracellular Ca2+ stores and ion channels in neurons. Trends in Neuroscience 23:80-88.

Faraguna U, Vyazovskiy VV, Nelson AB, Tononi G, Cirelli C (2008) A causal role for brain-derived neurotrophic factor in the homeostatic regulation of sleep. Journal of Neuroscience 28:4088-4095.

Feinberg I, Floyd TC (1979) Systematic trends across the night in human sleep cycles. Psychophysiology 16:283-291.

Feinberg I, Floyd TC, March JD (1987) Effects of sleep loss on delta (0.3-3 Hz) EEG and eye movement density: new observations and hypotheses. Electroencephalography and Clinical Neurophysiology 67:217-221.

Fellin T, Pascual O, Gobbo S, Pozzan T, Haydon PG, Carmignoto G (2004) Neuronal synchrony mediated by astrocytic glutamate through activation of extrasynaptic NMDA receptors. Neuron 43:729-743.

Ferre S, Karcz-Kubicha M, Hope BT, Popoli P, Burgueno J, Gutierrez MA, Casado V, Fuxe K, Goldberg SR, Lluis C, Franco R, Ciruela F (2002a) Synergistic interaction between adenosine A2A and glutamate mGlu5 receptors: implications for striatal neuronal function. Proceedings of the National Academy of Sciences USA 99:11940-11945.

Ferre S, Karcz-Kubicha M, Hope BT, Popoli P, Burgueno J, Gutierrez MA, Casado V, Fuxe K, Goldberg SR, Lluis C, Franco R, Ciruela F (2002b) Synergistic interaction between adenosine A2A and glutamate mGlu5 receptors: Implications for striatal neuronal function. Proceedings of the National Academy of Sciences of the United States of America 99:11940-11945.

Finelli LA, Achermann P, Borbely AA (2001a) Individual 'fingerprints' in human sleep EEG topography. Neuropsychopharmacology 25:S57-62.

Finelli LA, Borbely AA, Achermann P (2001b) Functional topography of the human nonREM sleep electroencephalogram. European Journal of Neuroscience 13:2282-2290.

Finelli LA, Baumann H, Borbely AA, Achermann P (2000) Dual electroencephalogram markers of human sleep homeostasis: correlation between theta activity in waking and slow-wave activity in sleep. Neuroscience 101:523-529.

Fontinha BM, Diogenes MJ, Ribeiro JA, Sebastiao AM (2008) Enhancement of long-term potentiation by brain-derived neurotrophic factor requires adenosine A2A receptor activation by endogenous adenosine. Neuropharmacology 54:924-933.

Francis LJ, Lewis CA, Ziebertz H (2006) The short-from revised Eysenck personality Questionnaire (EOQ-S): A German version. Socical Behavior and Personality 34:197-204.

Frank MG, Issa NP, Stryker MP (2001) Sleep enhances plasticity in the developing visual cortex. Neuron 30:275-287.

Franken P, Chollet D, Tafti M (2001) The homeostatic regulation of sleep need is under genetic control. Journal of Neuroscience 21:2610-2621.

Gallopin T, Luppi PH, Rambert FA, Frydman A, Fort P (2004) Effect of the wake-promoting agent modafinil on sleep-promoting neurons from the ventrolateral preoptic nucleus: an in vitro pharmacologic study.[see comment]. Sleep 27:19-25.

Gallopin T, Luppi PH, Cauli B, Urade Y, Rossier J, Hayaishi O, Lambolez B, Fort P (2005) The endogenous somnogen adenosine excites a subset of sleep-promoting neurons via A2A receptors in the ventrolateral preoptic nucleus. Neuroscience 134:1377-1390.

Gasparini F, Bilbe G, Gomez-Mancilla B, Spooren W (2008a) mGluR5 antagonists: discovery, characterization and drug development. Current opinion in drug discovery & development 11:655-665.

Gasparini F, Bilbe G, Gomez-Mancilla B, Spooren W (2008b) mGluR5 antagonists: Discovery, characterization and drug development. Current Opinion in Drug Discovery & Development 11:655-665.

Gerrard P, Malcolm R (2007) Mechanisms of modafinil: A review of current research. Journal of Neuropsychiatric Disease and Treatment 3:349-364.
Gilestro GF, Tononi G, Cirelli C (2009) Widespread changes in synaptic markers as a function of sleep and wakefulness in Drosophila. Science 324:109-112.
Guilleminault C, Brooks S (2001) Idiopathic hypersomnia: a neurological dilemma. Sleep Medicine Reviews 5:347-349.
Guilleminault C, Mignot E, Partinen M (1994) Controversies in the diagnosis of narcolepsy. Sleep 17:S1-6.
Hallanger AE, Levey AI, Lee HJ, Rye DB, Wainer BH (1987) The Origins of Cholinergic and Other Subcortical Afferents to the Thalamus in the Rat. Journal of Comparative Neurology 262:105-124.
Hayashi MK, Ames HM, Hayashi Y (2006) Tetrameric hub structure of postsynaptic scaffolding protein homer. Journal of Neuroscience 26:8492-8501.
Hays SA, Huber KM, Gibson JR (2011) Altered neocortical rhythmic activity states in Fmr1 KO mice are due to enhanced mGluR5 signaling and involve changes in excitatory circuitry. Journal of Neuroscience 31:14223-14234.
Horne JA (1993) Human sleep, sleep loss and behaviour. Implications for the prefrontal cortex and psychiatric disorder. British Journal of Psychiatry 162:413-419.
Horne JA, Östberg O (1976) A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. International Journal of Chronobiology 4:97-110.
Huber KM, Gallagher SM, Warren ST, Bear MF (2002) Altered synaptic plasticity in a mouse model of fragile X mental retardation. Proceeding of the National Academiy of Science USA 99:7746-7750.
Huber R, Deboer T, Tobler I (2000) Topography of EEG dynamics after sleep deprivation in mice. Journal of Neurophysiology 84:1888-1893.
Huber R, Ghilardi MF, Massimini M, Tononi G (2004) Local sleep and learning. Nature 430:78-81.
Huber R, Ghilardi MF, Massimini M, Ferrarelli F, Riedner BA, Peterson MJ, Tononi G (2006) Arm immobilization causes cortical plastic changes and locally decreases sleep slow wave activity. Nature Neuroscience 9:1169-1176.
Izumi Y, Zorumski CF (2012) NMDA receptors, mGluR5, and endocannabinoids are involved in a cascade leading to hippocampal long-term depression. Neuropsychopharmacology 37:609-617.
Izumi Y, Clifford DB, Zorumski CF (1991) 2-Amino-3-phosphonopropionate blocks the induction and maintenance of long-term potentiation in rat hippocampal slices. Neuroscience Letters 122:187-190.
Jasper HH (1958) Report of the committee on methods of clinical examination in electroencephalography. Appendix: The ten-twenty electrode system of the international federation. Electroencephalography and clinical neurophysiology 10:370-375.

Jia Z, Lu Y, Henderson J, Taverna F, Romano C, Abramow-Newerly W, Wojtowicz JM, Roder J (1998) Selective abolition of the NMDA component of long-term potentiation in mice lacking mGluR5. Learning & Memory 5:331-343.

Jones BE (1995) Reticular formation. Cytoarchitecture, transmitters and projections. In: In The Rat Nervous System, pp 155–171: Academic Press Australia.

Jones BE (2005a) From waking to sleeping: neuronal and chemical substrates. Trends in Pharmacological Sciences 26:578-586.

Jones BE (2005b) Basic mechanisms of sleep-wake states. In: Principles and Practise of Sleep Medicine, 4th Edition (Kryger MH, Roth T, Dement WC, eds), pp 136-153. Philadelphia, PA: Elsevier Saunders.

Jung SS, Sung KW, Lee SE, Shin HK (2011) Capsaicin prevents the hyperalgesia induced by peripheral group I mGluRs activation. Neuroscience Letters 500:197-201.

Kammermeier PJ, Worley PF (2007) Homer 1a uncouples metabotropic glutamate receptor 5 from postsynaptic effectors. Proceedings of the National Academy of Sciences USA 104:6055-6060.

Kamondi A, Acsady L, Wang XJ, Buzsaki G (1998) Theta oscillations in somata and dendrites of hippocampal pyramidal cells in vivo: activity-dependent phase-precession of action potentials. Hippocampus 8:244-261.

Kanbayashi T, Inoue Y, Chiba S, Aizawa R, Saito Y, Tsukamoto H, Fujii Y, Nishino S, Shimizu T (2002) CSF hypocretin-1 (orexin-A) concentrations in narcolepsy with and without cataplexy and idiopathic hypersomnia. Journal Sleep Research 11:91-93.

Kauer JA, Malenka RC (2007) Synaptic plasticity and addiction. Nature Reviews Neuroscience 8:844-858.

Kharazia VN, Phend KD, Rustioni A, Weinberg RJ (1996) EM colocalization of AMPA and NMDA receptor subunits at synapses in rat cerebral cortex. Neuroscience Letters 210:37-40.

Khatami R, Landolt HP, Achermann P, Rétey JV, Werth E, Mathis J, Bassetti CL (2007) Insufficient non-REM sleep intensity in narcolepsy-cataplexy. Sleep 30:980-989.

Khatami R, Landolt HP, Achermann P, Adam M, Rétey JV, Werth E, Schmid D, Bassetti CL (2008) Challenging sleep homeostasis in narcolepsy-cataplexy: Implications for nonREM and REM sleep regulation. Sleep 31:in press.

Klimesch W (1996) MEMORY PROCESSES, BRAIN OSCILLATIONS AND EEG SYNCHRONIZATION [Review]. International Journal of Psychophysiology 24:61-100.

Klimesch W (1999) EEG alpha and theta oscillations reflect cognitive and memory performance: a review and analysis. Brain Research Reviews 29:169-195.

Klintsova AY, Greenough WT (1999) Synaptic plasticity in cortical systems. Current Opinion in Neurobiology 9:203-208.

Knoblauch V, Martens WL, Wirz-Justice A, Cajochen C (2003) Human sleep spindle characteristics after sleep deprivation. Clinical Neurophysiology 114:2258-2267.

Krueger DD, Bear MF (2010) Toward fulfilling the promise of molecular medicine in fragile X syndrome. Annual Review of Medicine 62:411-429.

Krystal JH, Mathew SJ, D'Souza DC, Garakani A, Gunduz-Bruce H, Charney DS (2010) Potential psychiatric applications of metabotropic glutamate receptor agonists and antagonists. CNS Drugs 24:669-693.

Landolt HP (2008a) Genotype-dependent differences in sleep, vigilance, and response to stimulants. Current Pharmaceutical Design 14:3396-3407.

Landolt HP (2008b) Sleep homeostasis: A role for adenosine in humans? Biochemical Pharmacology 75:2070-2079.

Lavault S, Dauvilliers Y, Drouot X, Leu-Semenescu S, Golmard JL, Lecendreux M, Franco P, Arnulf I (2011) Benefit and risk of modafinil in idiopathic hypersomnia vs. narcolepsy with cataplexy. Sleep Medicine 12:550-556.

Legutko B, Szewczyk B, Pomierny-Chamiolo L, Nowak G, Pilc A (2006) Effect of MPEP treatment on brain-derived neurotrophic factor gene expression. Pharmacological Reports Online 58:427-430.

Lena I, Parrot S, Deschaux O, Muffat-Joly S, Sauvinet V, Renaud B, Suaud-Chagny MF, Gottesmann C (2005) Variations in extracellular levels of dopamine, noradrenaline, glutamate, and aspartate across the sleep--wake cycle in the medial prefrontal cortex and nucleus accumbens of freely moving rats. Journal of Neuroscience Research 81:891-899.

Lin L, Faraco R, Li H, Kadotani H, Rogers W, Lin X, Qui X, de Jong PJ, Nishino S, Mignot E (1999) The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell 98:365-376.

Liu ZW, Faraguna U, Cirelli C, Tononi G, Gao XB (2010) Direct evidence for wake-related increases and sleep-related decreases in synaptic strength in rodent cortex. Journal of Neuroscience 30:8671-8675.

Lu YM, Jia Z, Janus C, Henderson JT, Gerlai R, Wojtowicz JM, Roder JC (1997) Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. Journal of Neuroscience 17:5196-5205.

Majer M, Jones JF, Unger ER, Youngblood LS, Decker MJ, Gurbaxani B, Heim C, Reeves WC (2007) Perception versus polysomnographic assessment of sleep in CFS and non-fatigued control subjects: results from a population-based study. BMC Neurology 7:40.

Malenka RC, Bear MF (2004) LTP and LTD: an embarrassment of riches. Neuron 44:5-21.

Manahan-Vaughan D, Braunewell KH (2005) The metabotropic glutamate receptor, mGluR5, is a key determinant of good and bad spatial learning performance and hippocampal synaptic plasticity. Cerebral Cortex 15:1703-1713.

Maquet P, Degueldre C, Delfiore G, Aerts J, Péters JM, Luxen A, Franck G (1997) Functional neuroanatomy of human slow wave sleep. Journal of Neuroscience 17:2807-2812.

Maret S, Dorsaz S, Gurcel L, Pradervand S, Petit B, Pfister C, Hagenbuchle O, O'Hara BF, Franken P, Tafti M (2007) Homer1a is a core brain molecular correlate of sleep loss. Proceedings of the National Academy of Science USA 104:20090-20095.

Maron L, Rechtschaffen A, Wolpert EA (1964) Sleep cycle during napping. Archives of General Psychiatry 11:503-508.

Matsuura M, Yamamoto K, Fukuzawa H, Okubo Y, Uesugi H, Moriiwa M, Kojima T, Shimazono Y (1985) Age development and sex differences of various EEG elements in healthy children and adults--quantification by a computerized wave form recognition method. Electroencephalogr Clin Neurophysiol 60:394-406.

McNair D, Lorr M, Droppleman L (1981) EdITS Manual for the Profile of Mood States. San Diego, CA: EdITS.

Merica H, Blois R, Gaillard JM (1998) Spectral characteristics of sleep EEG in chronic insomnia. European Journal of Neuroscience 10:1826-1834.

Mignot E, Lammers GJ, Ripley B, Okun M, Nevsimalova S, Overeem S, Vankova J, Black J, Harsh J, Bassetti C, Schrader H, Nishino S (2002) The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. Archives of Neurology 59:1553-1562.

Mikolajczyk K, Szabatin M, Rudnicki P, Grodzki M, Burger C (1998) A JAVA environment for medical image data analysis: initial application for brain PET quantitation. Medical Information (Lond) 23:207-214.

Miller JD, Farber J, Gatz P, Roffwarg H, German DC (1983) Activity of mesencephalic dopamine and non-dopamine neurons across stages of sleep and walking in the rat. Brain Research 273:133-141.

Mochizuki T, Crocker A, McCormack S, Yanagisawa M, Sakurai T, Scammell TE (2004) Behavioral state instability in orexin knock-out mice. Journal of Neuroscience 24:6291-6300.

Montplaisir J, de Champlain J, Young SN, Missala K, Sourkes TL, Walsh J, Remillard G (1982) Narcolepsy and idiopthic hypersomnia: biogenic amines and related compounds in CSF. Neurology 32:1299-1302.

Moore RY, Eichler VB (1972) Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Research 42:201-206.

Moruzzi G, Magoun HW (1949) Brain stem reticular formation and activation of the EEG activation. Electroencephalography and clinical Neurophysiology 1:455-473.

Moses JM, Johnson LC, Naitoh P, Lubin A (1975) Sleep stage deprivation and total sleep loss: effects on sleep behavior. Psychophysiolgy 12:141-146.

Murphy M, Riedner BA, Huber R, Massimini M, Ferrarelli F, Tononi G (2009) Source modeling sleep slow waves. Proceedings of the National Academy in Science USA 106:1608-1613.

Nagahara AH, Tuszynski MH (2011) Potential therapeutic uses of BDNF in neurological and psychiatric disorders. Nature Reviews Drug Discovery 10:209-219.

Naie K, Manahan-Vaughan D (2004) Regulation by metabotropic glutamate receptor 5 of LTP in the dentate gyrus of freely moving rats: relevance for learning and memory formation. Cereb Cortex 14:189-198.

Naie K, Manahan-Vaughan D (2005) Pharmacological antagonism of metabotropic glutamate receptor 1 regulates long-term potentiation and spatial reference memory in the dentate gyrus of freely moving rats via N-methyl-D-aspartate and metabotropic glutamate receptor-dependent mechanisms. European Journal of Neuroscience 21:411-421.

Nelson DL, Cox MM (2008) G protein coupled receptor and second messanger. In: Lehninger Principles of Biochemistry, 5th Edition (Nelson DL, Cos, M.M, ed), pp 423-439. New York: WH Freemann and Company.

Neves SR, Ram PT, Iyengar R (2002) G protein pathways. Science 296:1636-1639.

Nevsimalova S, Blazejova K, Illnerova H, Hajek I, Vankova J, Pretl M, Sonka K (2000) A contribution to pathophysiology of idiopathic hypersomnia. Supplement Clinical Neurophysiology 53:366-370.

Niedermeyer E (2005) The normal EEG of the waking adult. In: Electroencephalography: Basic principles, clinical applications, and related fields, 5th Edition (Niedermeyer E, Lopes da Silva F, eds), pp 167-192. Philadelphia: Lippincott Williams & Wilkins.

Nir Y, Staba RJ, Andrillon T, Vyazovskiy VV, Cirelli C, Fried I, Tononi G (2011) Regional slow waves and spindles in human sleep. Neuron 70:153-169.

Nishino S, Mignot E (1997) Pharmacological aspects of human and canine narcolepsy. Progress in Neurobiology 52:27-78.

Nishino S, Mignot E (2005) Wake-promoting medications: Basic mechanisms and pharmacology. In: Principles and Practice of Sleep Medicine (Kryger MH, Roth T, Dement WC, eds), pp 468-483. Philadelphia, PA: Elsevier Saunders.

Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E (2000) Hypocretin (orexin) deficiency in human narcolepsy. Lancet 355:39-40.

Niswender CM, Conn PJ (2010) Metabotropic glutamate receptors: physiology, pharmacology, and disease. Annual Review of Pharmacology and Toxicology 50:295-322.

Nofzinger EA, Buysse DJ, Germain A, Price JC, Miewald JM, Kupfer DJ (2004) Functional neuroimaging evidence for hyperarousal in insomnia. American Journal of Psychiatry 161:2126-2128.

Okada T, Mochizuki T, Huang ZL, Eguchi N, Sugita Y, Urade Y, Hayaishi O (2003) Dominant localization of adenosine deaminase in leptomeninges and involvement of the enzyme in sleep. Biochemical & Biophysical Research Communications 312:29-34.

Olive MF (2009) Metabotropic glutamate receptor ligands as potential therapeutics for addiction. Current Drug Abuse Reviews 2:83-98.

Padamsey Z, Emptage NJ (2011) Imaging synaptic plasticity. Molecular Brain 4:36.

Perlis ML, Merica H, Smith MT, Giles DE (2001) Beta EEG activity and insomnia. Sleep Medicine Reviews 5:363-374.

Peyron C, Tighe DK, van den Pol AN, De Lecea L, Heller HC, Sutcliffe JG, Kilduff TS (1998) Neurons containing hypocretin (orexin) project to multiple neuronal systems. The Journal of Neuroscience 18:9996-100016.

Peyron C, Valentin F, Bayard S, Hanriot L, Bedetti C, Rousset B, Luppi PH, Dauvilliers Y (2011) Melanin concentrating hormone in central hypersomnia. Sleep Medicine 12:768-772.

Pin JP, Acher F (2002) The metabotropic glutamate receptors: structure, activation mechanism and pharmacology. CNS & Neurological Disorders - Drug Targets 1:297-317.

Rechtschaffen A, Kales A (1968) A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. Bethesda, Maryland: National Institutes of Health.

Rétey JV, Adam M, Honegger E, Khatami R, Luhmann UFO, Jung HH, Berger W, Landolt H-P (2005) A functional genetic variation of adenosine deaminase affects the duration and intensity of deep sleep in humans. Proceedings of the National Academy of Sciences of the United States of America 102:15676-15681.

Reymann KG, Matthies H (1989) 2-Amino-4-phosphonobutyrate selectively eliminates late phases of long-term potentiation in rat hippocampus. Neuroscience Letters 98:166-171.

Robbins T, Everitt B (1995) Central norepinephrine neurons and behavior in: F.E. Bloom, D.J. Kupfer (Eds.), Psychopharmacology: The Fourth Generation of Progress. Psychopharmacology:363-372.

Roediger HL, McDermott KB (1995) Creating False Memories: Remembering Words Not Presented in Lists. Journal of Experimental Psychology: Learning, Memory, and Cognition 21 803-814.

Romano C, Sesma MA, McDonald CT, O'Malley K, Van den Pol AN, Olney JW (1995) Distribution of metabotropic glutamate receptor mGluR5 immunoreactivity in rat brain. Journal of Comparative Neurology 355:455-469.

Ronesi JA, Huber KM (2008) Homer interactions are necessary for metabotropic glutamate receptor-induced long-term depression and translational activation. Journal of Neuroscience 28:543-547.

Ross EM (1989) Signal sorting and amplification through G protein-coupled receptors. Neuron 3:141-152.

Roth B (1976) Narcolepsy and hypersomnia: review and classification of 642 personally observed cases. Schweizer Archiv für Neurologie Neurochirurgie und Psychiatrie 119:31-41.

Roth T (2007) Insomnia: definition, prevalence, etiology, and consequences. Journal of Clinical Sleep Medicine 3:S7-10.

Rusterholz T, Durr R, Achermann P (2010) Inter-individual differences in the dynamics of sleep homeostasis. Sleep 33:491-498.

Sakurai T (2007) The neural circuit of orexin (hypocretin): maintaining sleep and wakefulness. Nature Reviews Neuroscience 8:171-181.

Sanacora G, Zarate CA, Krystal JH, Manji HK (2008) Targeting the glutamatergic system to develop novel, improved therapeutics for mood disorders. Nature Reviwes Drug Discovery 7:426-437.
Saper CB, Scammell TE, Lu J (2005) Hypothalamic regulation of sleep and circadian rhythms. Nature 437:1257-1263.
Saper CB, Fuller PM, Pedersen NP, Lu J, Scammell TE (2011) Sleep state switching. Neuron 68:1023-1042.
Schwartz JR (2004) Pharmacologic management of daytime sleepiness. Journal of Clinical Psychiatry 65 Suppl 16:46-49.
Sforza E, Gaudreau H, Petit D, Montplaisir J (2000) Homeostatic sleep regulation in patients with idiopathic hypersomnia. Clinical Neurophysiology 111:277-282.
Shiraishi-Yamaguchi Y, Furuichi T (2007) The Homer family proteins. Genome Biology 8:206.
Siegel JM (2004) The neurotransmitters of sleep. Journal of Clinical Psychiatry 65 Suppl 16:4-7.
Siegel SJ, Janssen WG, Tullai JW, Rogers SW, Moran T, Heinemann SF, Morrison JH (1995) Distribution of the excitatory amino acid receptor subunits GluR2(4) in monkey hippocampus and colocalization with subunits GluR5-7 and NMDAR1. Journal of Neuroscience 15:2707-2719.
Silva AJ (2003) Molecular and cellular cognitive studies of the role of synaptic plasticity in memory. Journal of Neurobiology 54:224-237.
Somers VK, Dyken ME, Mark AL, Abboud FM (1993) Sympathetic-nerve activity during sleep in normal subjects. The New England Journal of Medicine 328:303-307.
Spielberger CD, Gorsuch RL, Lushene RE (1970) Manual for the State-Trait Anxiety Inventory. Palo Alto, Calif.: Consulting Psychologists Press.
Stephan FK, Zucker I (1972) Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. Proceedings of the National Academy of Sciences USA 69:1583-1586.
Steriade M (1996) Arousal: revisiting the reticular activating system. Science 272:225-226.
Steriade M, Gloor P, Llinas RR, Lopes da Silva FH, Mesulam M-M (1990) Basic mechanisms of cerebral rhythmic activities. Electroencephalography and Clinical Neurophysiology [Zü65] 76:481-508.
Swanson CJ, Bures M, Johnson MP, Linden AM, Monn JA, Schoepp DD (2005) Metabotropic glutamate receptors as novel targets for anxiety and stress disorders. Nature Reviews Drug Discovery 4:131-144.
Szymusiak R, Alam N, Steininger TL, McGinty D (1998) Sleep-waking discharge patterns of ventrolateral preoptic/anterior hypothalamic neurons in rats. Brain Research 803:178-188.
Tadavarty R, Rajput PS, Wong JM, Kumar U, Sastry BR (2011) Sleep-deprivation induces changes in GABA(B) and mGlu receptor expression and has consequences for synaptic long-term depression. PLoS One 6:e24933.

Tafti M (2009) Genetic aspects of normal and disturbed sleep. Sleep Medicine 10 Supplement 1:S17-21.

Takei Y, Komada Y, Namba K, Sasai T, Nakamura M, Sugiura T, Hayashida K, Inoue Y (2012) Differences in findings of nocturnal polysomnography and multiple sleep latency test between narcolepsy and idiopathic hypersomnia. Clinical Neurophysiology 123:137-147.

Tebano MT, Martire A, Rebola N, Pepponi R, Domenici MR, Gro MC, Schwarzschild MA, Chen JF, Cunha RA, Popoli P (2005) Adenosine A2A receptors and metabotropic glutamate 5 receptors are co-localized and functionally interact in the hippocampus: a possible key mechanism in the modulation of N-methyl-D-aspartate effects. Journal of Neurochemistry 95:1188-1200.

Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, Cornford M, Siegel JM (2000) Reduced number of hypocretin neurons in human narcolepsy. Neuron 27:469-474.

Tononi G, Cirelli C (2001) Some considerations on sleep and neural plasticity. Archives Italiennes de Biologie 139:221-241.

Tononi G, Cirelli C (2003) Sleep and synaptic homeostasis: a hypothesis. Brain Research Bulletin 62:143-150.

Tononi G, Cirelli C (2006) Sleep function and synaptic homeostasis. Sleep Medicine Reviews 10:49-62.

Torrealba F, Yanagisawa M, Saper CB (2003) Colocalization of orexin a and glutamate immunoreactivity in axon terminals in the tuberomammillary nucleus in rats. Neuroscience 119:1033-1044.

Tracey I (2008) Imaging pain. British Journal of Anaesthesia 101:32-39.

Treyer V, Streffer J, Wyss MT, Bettio A, Ametamey SM, Fischer U, Schmidt M, Gasparini F, Hock C, Buck A (2007) Evaluation of the metabotropic glutamate receptor subtype 5 using PET and 11C-ABP688: assessment of methods. Journal of Nuclear Medicine 48:1207-1215.

Treyer V, Streffer J, Ametamey SM, Bettio A, Blauenstein P, Schmidt M, Gasparini F, Fischer U, Hock C, Buck A (2008) Radiation dosimetry and biodistribution of 11C-ABP688 measured in healthy volunteers. European Journal of Nuclear Medicine and Molecular Imaging 35:766-770.

Tronson NC, Guzman YF, Guedea AL, Huh KH, Gao C, Schwarz MK, Radulovic J (2010) Metabotropic glutamate receptor 5/Homer interactions underlie stress effects on fear. Biological Psychiatry 68:1007-1015.

Tsanov M, Manahan-Vaughan D (2009) Long-term plasticity is proportional to theta-activity. PLoS One 4:e5850.

Tu JC, Xiao B, Yuan JP, Lanahan AA, Leoffert K, Li M, Linden DJ, Worley PF (1998) Homer binds a novel proline-rich motif and links group 1 metabotropic glutamate receptors with IP3 receptors. Neuron 21:717-726.

Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B, Joliot M (2002) Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. Neuroimage 15:273-289.

Urade Y, Eguchi N, Qu W-M, Sakata M, Huang ZL, Chen JF, Schwarzschild MA, Fink JS, Hayaishi O (2003) Sleep regulation in adenosine A_{2A} receptor-deficient mice. Neurology 61:S94-S96.

van Beijsterveldt CE, Molenaar PC, de Geus EJ, Boomsma DI (1996) Heritability of human brain functioning as assessed by electroencephalography. American Journal of Human Genetics 58:562-573.

Van Cauter E, Leproult R, Kupfer DJ (1996) Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. Journal of Clinical Endocrinology and Metabolism 81:2468-2473.

van den Pol AN, Romano C, Ghosh P (1995) Metabotropic glutamate receptor mGluR5 subcellular distribution and developmental expression in hypothalamus. Journal of Comparative Neurology 362:134-150.

Van Dongen HP, Maislin G, Mullington JM, Dinges DF (2003) The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. Sleep 26:117-126.

Vankova J, Nevsimalova S, Sonka K, Spackova N, Svejdova-Blazejova K (2001) Increased REM density in narcolepsy-cataplexy and the polysymptomatic form of idiopathic hypersomnia. Sleep 24:707-711.

Vernet C, Leu-Semenescu S, Buzare MA, Arnulf I (2010) Subjective symptoms in idiopathic hypersomnia: beyond excessive sleepiness. Journal of Sleep Research 19:525-534.

Viola AU, Archer SN, James LM, Groeger JA, Lo JC, Skene DJ, von Schantz M, Dijk DJ (2007) PER3 polymorphism predicts sleep structure and waking performance. Current Biology 17:613-618.

Viwatpinyo K, Chongthammakun S (2009) Activation of group I metabotropic glutamate receptors leads to brain-derived neurotrophic factor expression in rat C6 cells. Neuroscience Letters 467:127-130.

Volkow ND, Tomasi D, Wang GJ, Telang F, Fowler JS, Logan J, Benveniste H, Kim R, Thanos PK, Ferré S (2012) Evidence That Sleep Deprivation Downregulates Dopamine D2R in Ventral Striatum in the Human Brain. Journal of Neuroscience 32:6711-6717.

Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Wong C, Ma J, Pradhan K, Tomasi D, Thanos PK, Ferre S, Jayne M (2008) Sleep deprivation decreases binding of [11C]raclopride to dopamine D2/D3 receptors in the human brain. Journal of Neuroscience 28:8454-8461.

Volkow ND, Fowler JS, Logan J, Alexoff D, Zhu W, Telang F, Wang GJ, Jayne M, Hooker JM, Wong C, Hubbard B, Carter P, Warner D, King P, Shea C, Xu Y, Muench L, Apelskog-Torres K (2009) Effects of modafinil on dopamine and dopamine transporters in the male human brain: clinical implications. Jama 301:1148-1154.

Vyazovskiy VV, Cirelli C, Pfister-Genskow M, Faraguna U, Tononi G (2008) Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. Nature Neuroscience 11:200-208.

Vyazovskiy VV, Olcese U, Hanlon EC, Nir Y, Cirelli C, Tononi G (2011) Local sleep in awake rats. Nature 472:443-447.

Walter WG, Dovey VJ (1944) Electro-Encephalography in Cases of Sub-Cortical Tumour. J Neurol Neurosurg Psychiatry 7:57-65.
Webb WB, Agnew HW, Jr. (1971) Stage 4 sleep: influence of time course variables. Science 174:1354-1356.
Weber JM, Schwander JC, Unger I, Meier D (1997) A direct ultrasensitive RIA for the determination of melatonin in thuman saliva: comparison with serum levels. Journal of Sleep Research 26:757.
Weyers P, Krebs H, Janke W (1995) Reliability and construct-validity of the German version of Cloninger's Tridimensional Personality Questionnaire. Personality and Individual Differences 19:853-861.
Worley PF, Zeng W, Huang G, Kim JY, Shin DM, Kim MS, Yuan JP, Kiselyov K, Muallem S (2007) Homer proteins in Ca2+ signaling by excitable and non-excitable cells. Cell Calcium 42:363-371.
Xiao B, Tu JC, Worley PF (2000) Homer: a link between neural activity and glutamate receptor function. Current Opinion in Neurobiology 10:370-374.
Xu J, Zhu Y, Contractor A, Heinemann SF (2009) mGluR5 has a critical role in inhibitory learning. Journal of Neuroscience 29:3676-3684.
Yan QJ, Rammal M, Tranfaglia M, Bauchwitz RP (2005) Suppression of two major Fragile X Syndrome mouse model phenotypes by the mGluR5 antagonist MPEP. Neuropharmacology 49:1053-1066.
Ying SW, Futter M, Rosenblum K, Webber MJ, Hunt SP, Bliss TV, Bramham CR (2002) Brain-derived neurotrophic factor induces long-term potentiation in intact adult hippocampus: requirement for ERK activation coupled to CREB and upregulation of Arc synthesis. Journal of Neuroscience 22:1532-1540.
Young EA, Carlson NE, Brown MB (2001) Twenty-four-hour ACTH and cortisol pulsatility in depressed women. Neuropsychopharmacology 25:267-276.
Yuste R, Bonhoeffer T (2001) Morphological changes in dendritic spines associated with long-term synaptic plasticity. Annual Review in Neuroscience 24:1071-1089.
Zhou Z, Zhen J, Karpowich NK, Goetz RM, Law CJ, Reith ME, Wang DN (2007) LeuT-desipramine structure reveals how antidepressants block neurotransmitter reuptake. Science 317:1390-1393.

Appendix

I Supplementary information to chapter 1

Methodology of Positron emission tomography (PET)

PET is a non-invasive nuclear imaging method which enables the visualization of cellular function and molecular processes in the living organism under physiological and pathophysiological conditions. For example, using an appropriate radiotracer a so-called biodistribution study can be performed to study the occurrence or absence of a specific molecule or receptor in specific organs or tissue regions.

Instable atoms which decay are referred to as radionuclides. Radionuclides used in PET scanning are isotopes emitting positrons (β+-decay) and typically have short half-live times ($t_{1/2}$), such as carbon-11 (^{11}C; $t_{1/2}$ = 20.4min), nitrogen-13 (^{13}N; $t_{1/2}$ = 9.9min), oxygen-15 (^{15}O; $t_{1/2}$ = 2.04min) or fluorine-18 (^{18}F; $t_{1/2}$ = 110min). These radionuclides, which are isotopes of the biologically ubiquitous elements, are incorporated either into compounds normally used by the body such as glucose, water or into molecules that bind to receptors. Such labeled compounds are known as radiotracers. Due to the short half-live time of the isotopes, the cyclotron for the production of these radiotracers must be in close proximity to the PET imaging facility.

A big advantage of PET imaging is that only little tracer amounts of a given radiotracer have to be injected for appropriate detection efficiency. Therefore, no physiological effects are expected. After intravenous injection, the concentration of the radiotracer in tissue is measured by the PET scanner in a static manner. During the decay process, the radionuclide emits a positively charged positron (e+, positive β-decay). After traveling a short distance (in the range of few millimeters) it encounters an electron (e^-) from the surrounding tissue. The

encounter annihilates both electron and positron resulting in the emission of a pair of γ-rays each of 511keV moving in approximately opposite direction. The image acquisition is based on the coincidence detection of the emitted γ–rays. Two detectors on the opposite side of the scanner reconstruct and produce an image with region specific density or activity of the target molecule. After image acquisition, attenuation correction is performed by applying mathematical algorithms. The reason for this correction is the differential attenuation of the photons by the tissue. Structures deep in the body are reconstructed as having falsely low tracer uptake. Contemporary PET/CT scanners estimate attenuation based on the CT scan acquired in the same imaging session.

Even though PET is a non-invasive method it does involve exposure to ionizing radiation. The total dose of radiation is substantial, usually around 5-7mSv per PET scan. Nowadays, often a combined PET/CT scan is performed and the radiation exposure may increase up to 23-26mSv for a 70kg person. (The sievert (Sv) is the unit of dose equivalent radiation. It attempts to quantitatively evaluate the biological effects of ionizing radiation as opposed to the physical aspects, which are characterised by the absorbed does, measured in gray (Gy). The unit Gy measures the absorbed dose of radiation, absorbed by any material. In contrast, Sv measures the equivalent dose of radiation supposed to have a damaging effect equivalent to the same dose of γ rays)

Compared with other non-invasive imaging techniques PET has a high sensitivity with moderate temporal and spatial resolution (Fig. I).

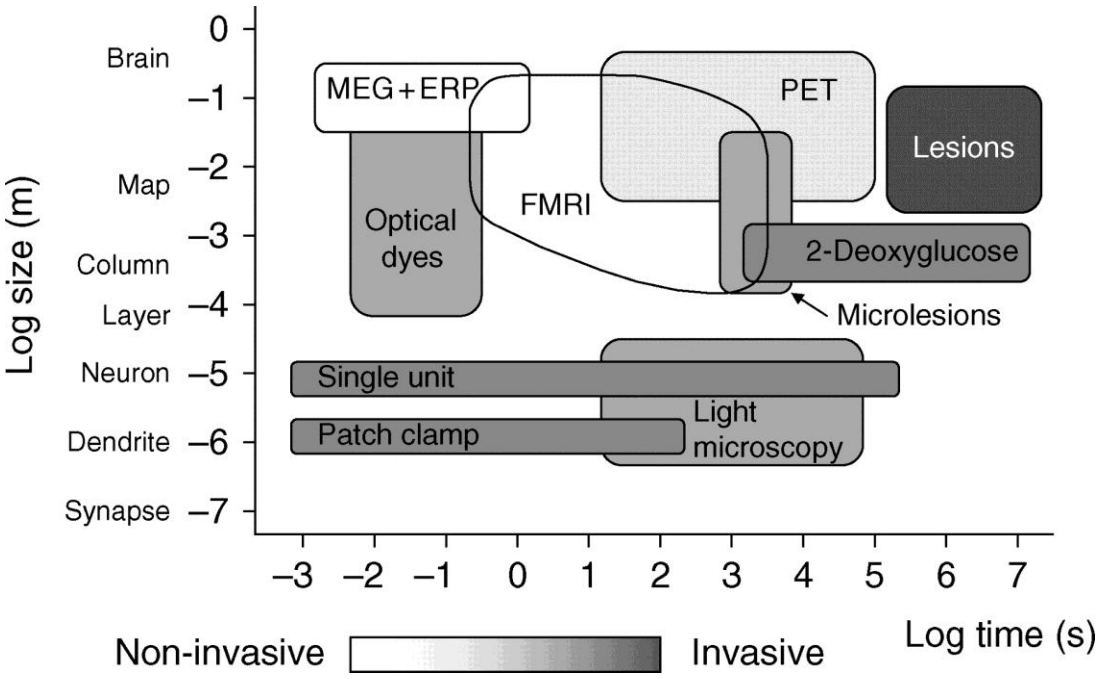


Figure I: A schema displaying the relationship between the spatial and temporal resolution in terms of their invasiveness for commonly used imaging tools. Axes represent logarithmic values (Tracey, 2008).

3-(6-methyl-pyridin-2-ylethynyl)-cyclohex-2-enone O-methyl-oxime (^{11}C-ABP688)

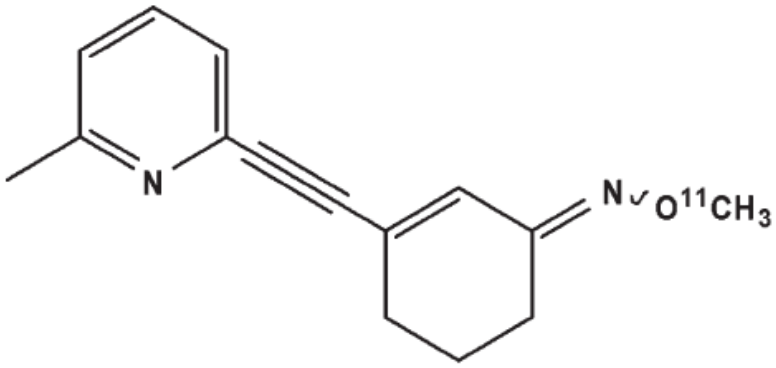


Figure II: Structure of ^{11}C-ABP688 (Ametamey et al., 2007).

One of the most commonly used radionuclide is C^{11} since it can be incorporated into many molecules without significant effect on biological activity of the molecule and in addition, the short half life allows repeated injections in the same

subject and day. ^{11}C-ABP688 was recently developed in collaboration between Novartis and the ETH Zürich and is the first radioligand for imaging the mGluR5 in vivo. Radiosynthesis was performed as described by Ametamey et al. (Ametamey et al., 2007). ^{11}C-ABP688 exhibits a high in vitro and in vivo affinity as well as a high specificity for mGluR5 and was found to have a saturation level of receptor binding (Ametamey et al., 2006). It binds to the allosteric site of the mGluR5.

The key properties of a good radioligand for receptor imaging and for high quality PET images are high specific radioactivity (SA) to have negligible receptor occupancy by non-radioactive compound; the selectivity over other receptors depends upon both the affinity of the tracer as well as the quantity of non-specific binding. Ametamey et al. (Ametamey et al., 2006) reported that the specificity of ^{11}C-ABP688 binding was confirmed by blockade studies with M-MPEP an antagonist for mGluR5. Up to 80% specific binding was observed for the hippocampus and striatum, regions with known high mGluR5 densitiy. No blocking effects were observed in the cerebellum. The data suggest that ^{11}C-ABP688 has a favorable metabolic profile.

Although the radiolabeled metabolites were not individually identified, the metabolite profile suggests a similar metabolic fate of ^{11}C-ABP688 in both, rodents and humans (Ametamey et al., 2007). Animal studies in wild type mice and rats showed in ex vivo autoradiography the highest uptake in hippocampus, striatum and the cortex (Ametamey et al., 2006). Biodistribution study in humans by Treyer et al. (Treyer et al., 2008) proclaimed that mGluR5 is a promising ligand to study mGluR5 in humans because the brain uptake is high and the effective dose equivalent so low making serial examinations in the same subject feasible.

In humans, the rich mGluR5 regions are the anterior cingulated cortex (ACC), medial temporal lobe, amygdale, caudate and putamen. Regions with little mGluR5 density are the cerebellum and the white matter. The highest uptake

was measured in the ACC with 5.45 ± 1.47 (arbitrary unit) and the lowest in cerebellum with 1.91 ± 0.32 (Ametamey et al., 2007).

The observations that mGluR5 may play an important role in a wide range of mental disorders, such as anxiety disorders, depression etc. made it a target of high interest in psychiatry research. One study with depressed patients showed a decreased mGluR5 density compared with healthy control subjects (Deschwanden et al., 2011). Furthermore, this tracer may be used in the future to study and evaluate drug-induced receptor occupancy, as well as in detecting possible differences in brain mGluR5 receptor levels of different psychiatric disorders.

Image acquisition and processing

The volunteers were prepared with two antecubital vein catheters for tracer injection on one side and for blood sampling on the other side .
Before PET scanning started a low-dose CT scan was acquired for correction of photon attenuation. The method used is a bolus-infusion (B/I) protocol. It is useful for fast receptor binding. During the first two minutes half of the radiotracer is injected via a bolus, while during the following 58 minutes the second half is infused continuously. Treyer et al. (2007) showed that after 40 minutes an equilibrium should be achieved which directly represents the distribution volume (Fig. III) of the mGluR5. Image acquisition was started a the same timepoint as the tracer injection. The injected doses of ^{11}C-ABP688, measured in Bequerel (Bq) normally lied between 477-746 kBq/cc. Every minute during the first ten minutes and every five minutes in the following 50 minutes an image was acquired resulting in a static data set of 20 images. Blood samples were taken after 58 minutes for metabolite determination. An aliquot of the sample was measured in a y-counter. Due to the recent publication of Burger et al. (Burger et al., 2010) where they showed that with the B/I protocol early time points correspond to perfusion and later time points to the total distribution volume, we

decided to use the average over the late frames (45, 50, 55 minutes) to obtain the uptake pattern and as a representative value of the DV.

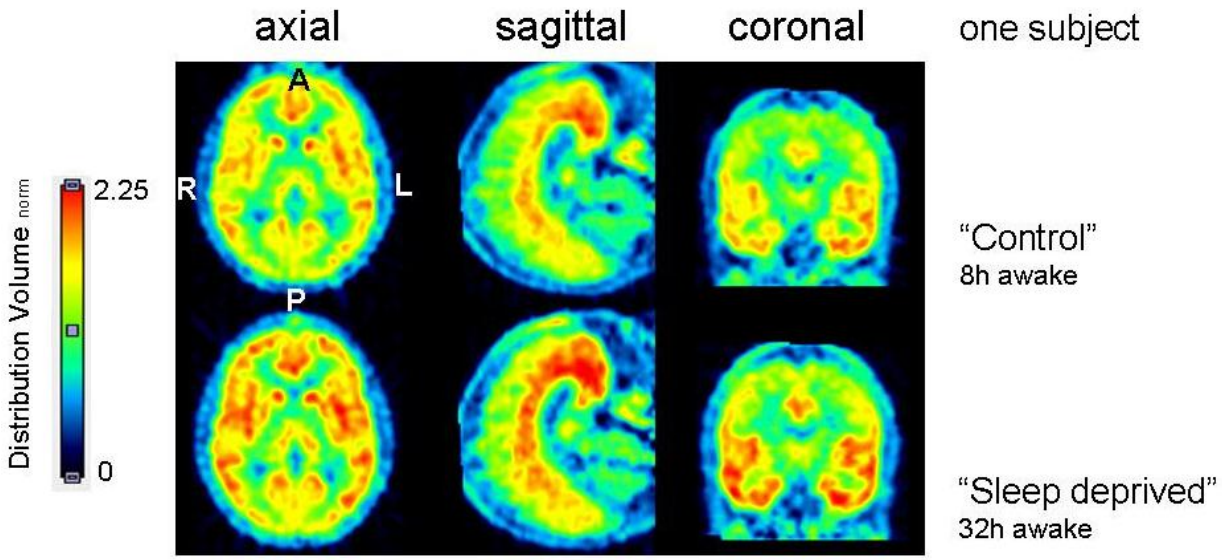


Figure III: Normalized distribution volume of one subject.
Top row images of control condition; bottom row images of sleep deprived condition.

All calculations were performed using the dedicated software PMOD (PMOD Technologies) (Mikolajczyk et al., 1998). After averaging the images, rigid matching was used for the comparison of the images from the control and sleep deprived condition. Furthermore, the images needed to be transformed into a reference space (Montreal Neurological Institute, MNI) to make comparison between different individuals possible. In a next step, the whole brain activity was divided by the cerebellum activity (cerebellum is suggested to be a valuable reference region because of very low receptor binding). The cerebellum was encircled by hand for each person and overlaid with the PET image and divided by the cerebellum value. In addition, MRI was performed to exclude any cerebral pathologies. As a last step, volume of interest (VOI) analysis was performed. The VOI analysis was based on the standard VOIs defined by the automated anatomical labelling (AAL) template (Tzourio-Mazoyer et al., 2002). Figure V represents a template of two regions.

The VOIs were chosen according to literature and included: Superior medial frontal cortex, orbitofrontal cortex, anterior cingulat cortex, insula, middle temporal cortex, precuneus, striatum, amygdale, hippocampus, parahippocampus, thalamus and an average group value of all brain regions excluding the cerebellum for both hemispheres separate.

To justify the findings two more variables needed to be generated. First, the time activity curve (TAC), to verify that in all subjects the equilibrium, was reached. Four regions were chosen: the putamen (left and right), frontal superior cortex, (left and right), thalamus (left and right) and the cerebellum as a whole. The resulting TACs confirmed in all regions and subjects a steady state in the second half of the scan (Fig. IV).

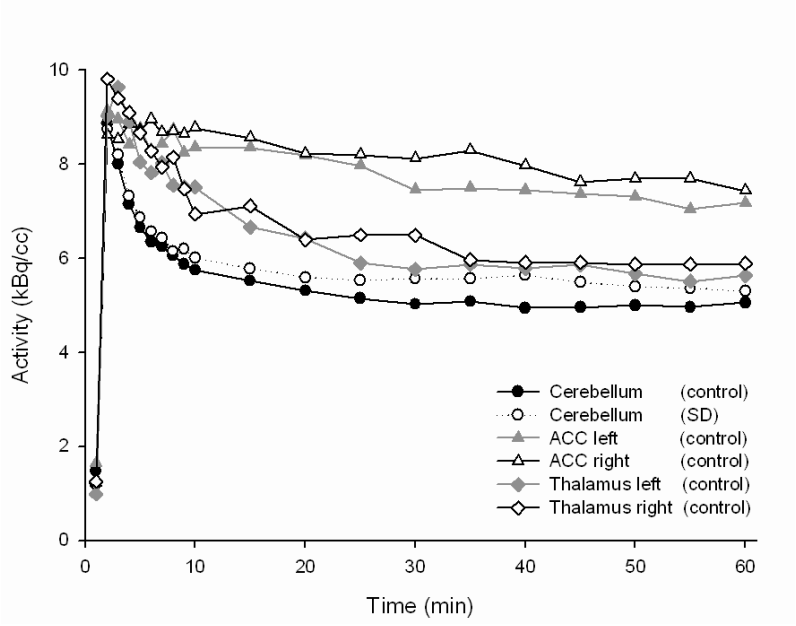


Figure IV: Representative time activity curves of ^{11}C-ABP688 uptake in a single subject.
The curve displays the evolution of a bolus-infusion protocol. Each symbol represents a time point where a picture was taken. The first ten images have an interval of one minute whereas the following ten images have an in-between interval of five minutes. Relative high radioactivity uptake is observed in mGluR5 rich regions such as the anterior cingulated cortex (ACC), moderate binding in the thalamus and low uptake in the cerebellum.
Solid line represents data from control condition whereas the dotted line represents data from sleep deprived condition.

Second, the standard uptake value (SUV) of cerebellum needed to be calculated to show that the intervention, in our case the sleep deprivation, had no effect on the receptor availability in the cerebellum. Otherwise, cerebellum could not be used as the reference region for the normalisation. The radioactivity concentrations of the cerebellum were decay-corrected and normalized to injected radioactivity dose and body weight. The results revealed no difference in the cerebellum mGluR5 density between the two conditions.

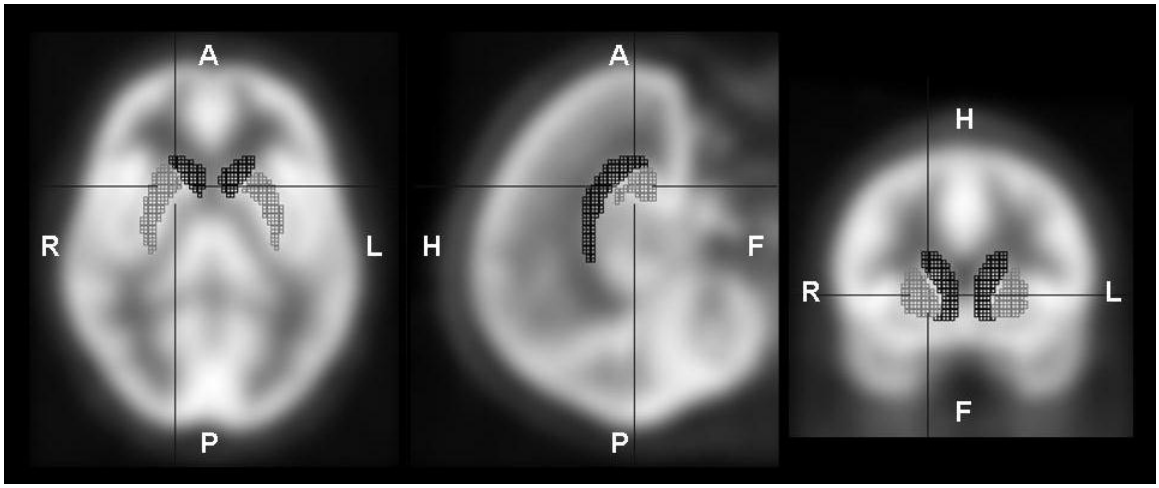
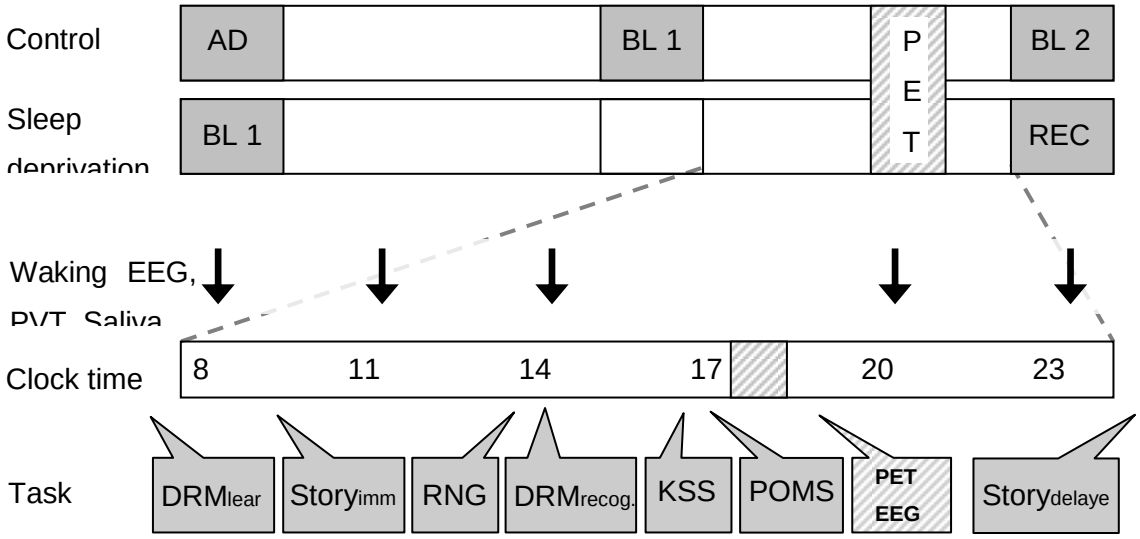


Figure V: Region of interest according to PMOD atlas.

Black: caudate; Grey: putamen; Images in axial, sagittal and coronal view; R: right side; L: left side; A: Anterior; P: Posterior; Grey: Putamen right hemisphere; White: Caudate right hemisphere.

II Supplementary information to chapter 2

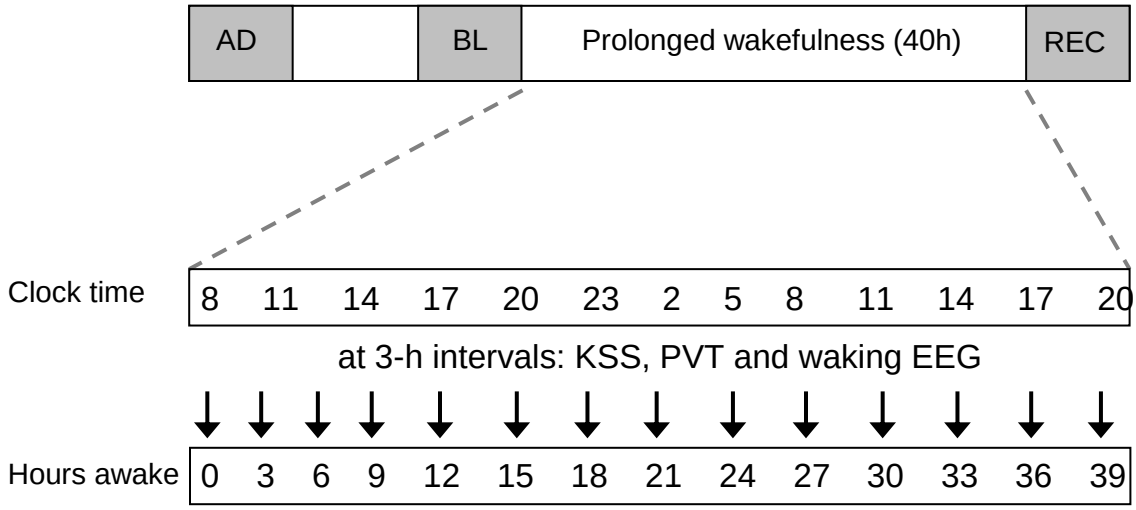
Study design of the mGluR5 project



All subjects participated in a two weeks randomized and cross-over study protocol. Sleep in adaptation (AD), baseline (BL) and recovery (REC) night was monitored with polysomnographically recordings. A positron emission tomography (PET) was measured at 4 pm after 9 or 33 hours awake respectively. During the 40 hours of prolonged wakefulness and during the second day during the control week different cognitive tasks and questionnaires needed to be filled in. DRM: Deese-Roedinger-McDermott memory task; Story: listening to a story and recall it immediately and at a delayed time point; RNG: Random Number Generation task; KSS: Karolinska Sleepiness Scale; POMS: Profile Of Mood State; EEG: 8 minutes electroencephalogram during wakefulness.

III Supplementary information to chapter 3

Study design of the idiopathic hypersomnia project



Idiopathic hypersomnia patients and healthy matched controls participated in a study design including a period of 40 hours without sleep. Sleep in adaptation (AD), baseline (BL) and recovery (REC) night was monitored by polysomnographically recordings. During the period of prolonged wakefulness patients and controls filled in questionnaires, performed tasks and EEG were measured in 3-hour intervals. KSS: Karolinska Sleepiness Scale; PVT: Psychomotor Vigilance task; EEG: 8-minutes electroencephalogram recording during wakefulness.

Curriculum Vitae

Katharina Hefti

Date of Birth	April 4, 1983
Place of Birth	Brugg (AG), Switzerland
Citizenship	Luchsingen (GL), Switzerland

Education and Training Periods

10/2008 – 05/2012	Doctoral Student in the Research Section of Chronobiology and Sleep Research in the Institute of Pharmacology and Toxicology at the University of Zürich, Switzerland
10/2008 – 05/2012	Student in the International PhD Program in Neuroscience, Neuroscience Center Zürich (ZNZ), Switzerland
2009 – 2010	ESRS-EU «Marie Curie» - PENS Training Program Training in Sleep Research and Sleep Medicine “Special Topic: Sleep and Memory” Bertinoro, Italy, 8-13 May 2009 University of Surrey, UK, 23-28 November 2009 Seeon, Germany, 2-6 July 2010
10/2007 – 08/2008	Master of Science in Human Biology, University of Zürich Switzerland
09/2004 – 08/2007	Bachelor of Science in Biology, University of Zürich, Switzerland
08/2000 – 06/2004	High School, Baden, Switzerland Specialization: Biology and Chemistry Supplementary courses: Sport and Anatomy Introduction to: Humanities and Social Sciences

List of publications

Katharina Hefti, Sebastian C. Holst, Judit Sovago, Valérie Bachmann, Alfred Buck, Simon M. Ametamey, Milan Scheidegger, Thomas Berthold, Baltazar Gomez-Mancilla, Erich Seifritz, and Hans-Peter Landolt (2012)
Increased availability of metabotropic glutamate receptor subtype 5 in human brain after one night without sleep. Biological Psychiatry, in revision

Oral presentations at national and international meetings

Progress Report Seminar, Institute of Pharmacology and Toxicology, University of Zürich, March 2009
"Sleep and waking EEG in patients with neurological disorders"

ZNZ PhD-Retreat, Valens, Mai 2009
"Sleep-waking regulation in patients with neurological disorders"

Progress Report Seminar, Institute of Pharmacology and Toxicology, University of Zürich, December 2009
"Sleep-wake regulation in patients with idiopathic hypersomnia"

Monday Group Meeting, Institute of Pharmacology and Toxicology, University of Zürich, April 2010
"mGluR5 and sleep homeostasis in humans: A positron emission tomography (PET) study"

Progress Report Seminar, Institute of Pharmacology and Toxicology, University of Zürich, June 2010
"mGluR5 and sleep homeostasis in humans: A positron emission tomography (PET) study"

20th Congress of the European Sleep Research Society, Lisbon, Portugal, September 2010

"mGluR5 and sleep homeostasis in humans: A positron emission tomography (PET) study"

Internal Research meeting, Department of Neurology, University Hospital Zürich, December 2010

"Sleep-wake regulation in patients with idiopathic hypersomnia"

Progress Report Seminar, Institute of Pharmacology and Toxicology, University of Zürich, March 2011

"mGluR5 and sleep homeostasis in humans: A positron emission tomography (PET) study"

Brain Fair 2011, Privatschule Lernstudio, Zürich, March 2011

"Schlaf"

Annual Conference of the Swiss Society of Sleep Research, Sleep Medicine and Chronobiology & Swiss Neurological Society, St.Gallen, November 2011

"Prolonged wakefulness increases metabotropic glutamate receptor 5 availability in human brain"

Internal Research meeting, Department of Paraplegiology, University Hospital Balgrist, December 2011

"Studien in der Schlafforschung in Bezug auf para/tetraplegische Patienten"

Poster presentations at national and international meetings

Annual Conference of the Swiss Society of Sleep Research, Sleep Medicine and Chronobiology & Swiss Society of Biological Psychiatry, Bern, March 2009
Hefti K, van Hedel H, Dietz V and Landolt H.-P.
"Sleep and waking EEG in patients with tetraplegia: Preliminary findings"

8th Day of Clinical Research, University Hospital, Zürich, April 2009
Hefti K, van Hedel H, Dietz V and Landolt H.-P.
"Sleep and waking EEG in patients with tetraplegia: Preliminary findings"

Pharma Poster Day, University of Zürich, July 2009
Hefti K, van Hedel H, Dietz V and Landolt H.-P.
"Sleep and waking EEG in patients with tetraplegia: Preliminary findings"

5th ZIHP Symposium, University Zürich, August 2009
Hefti K, Khatami R, Nadig U, Poryazova R, Högl B, Bassetti C and Landolt H-P
"Sleep-wake regulation in idiopathic hypersomnia"

9th Day of Clinical Research, University Hospital Zürich, April 2010
Hefti K, Khatami R, Nadig U, Poryazova R, Högl B, Bassetti C and Landolt H-P
"Sleep-wake regulation in idiopathic hypersomnia"

Annual Conference of the Swiss Society of Sleep Research, Sleep Medicine and Chronobiology & Swiss Society of Neuroscience, Lausanne, March 2010
Hefti K, Khatami R, Nadig U, Poryazova R, Högl B, Bassetti C and Landolt H-P
"Sleep homeostatis in patients with idiopathic hypersomnia"

Zürich PHARMA/TOX Poster Day, University Zürich, June 2010
Hefti K, Khatami R, Nadig U, Poryazova R, Högl B, Bassetti C and Landolt H-P
"Sleep homeostatis in patients with idiopathic hypersomnia"

6th ZIHP Symposium, University Zürich, August 2010

Hefti K, Khatami R, Nadig U, Poryazova R, Högl B, Bassetti C and Landolt H-P

"Sleep homeostatis in patients with idiopathic hypersomnia"

20th Congress of the European Sleep Research Society, Lisbon, Portugal, September 2010

Hefti K, Wehrle R, Sovago J, Treyer V, Bachmann V, Berthold T, Buck A, Ametamey S. and Landolt H.-P.

"Metabotropic glutamate receptor subtype 5 (mGluR5) density and sleep-wake regulation: A positron emission tomography study"

Pharmacology Poster Day, University Zürich, June 2011

Hefti K, Wehrle R, Sovago J, Treyer V, Bachmann V, Berthold T, Buck A, Ametamey S. and Landolt H.-P.

"Metabotropic glutamate receptor subtype 5 (mGluR5) density and sleep-wake regulation: A positron emission tomography study"

7th ZIHP Symposium, University Zürich, August 2011

Hefti K, Wehrle R, Sovago J, Treyer V, Bachmann V, Berthold T, Buck A, Ametamey S. and Landolt H.-P.

"Prolonged wakefulness increases mGluR5 density in the human brain: Association with impaired vigilance and cognitive performance"

ZNZ Symposium, University Zürich, September 2011

Hefti K, Wehrle R, Sovago J, Treyer V, Bachmann V, Berthold T, Buck A, Ametamey S. and Landolt H.-P.

"Prolonged wakefulness increases mGluR5 density in the human brain: Association with impaired vigilance and performance"

11th Day of Clinical Research, University Hospital Zürich, April 2012

Hefti K, Khatami R, Nadig U, Poryazova R, Högl B, Bassetti C and Landolt H-P

"Reduced EEG β-activity in idiopathic hypersomnia in sleep and wakefulness"

Award for best free communication

Joint Annual Meeting of the SSSSC and SNS 2011, St.Gallen, 4.November 2011

“Prolonged wakefulness increases metabotropic glutamate receptor 5 availability in human brain”

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Katharina von Siena 1347-1380